



US008344120B2

(12) **United States Patent**
Mjalli et al.

(10) **Patent No.:** **US 8,344,120 B2**
(45) **Date of Patent:** **Jan. 1, 2013**

(54) **NUCLEIC ACID MOLECULES ENCODING RAGE FUSION PROTEINS**

(75) Inventors: **Adnan M. M. Mjalli**, Oak Ridge, NC (US); **Robert Rothlein**, Summerfield, NC (US); **Ye Edward Tian**, Jamestown, NC (US); **Jeffrey C. Webster**, Jamestown, NC (US)

(73) Assignee: **TransTech Pharma, Inc.**, High Point, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/158,748**

(22) Filed: **Jun. 13, 2011**

(65) **Prior Publication Data**

US 2011/0282035 A1 Nov. 17, 2011

Related U.S. Application Data

(62) Division of application No. 11/789,637, filed on Apr. 25, 2007, now Pat. No. 7,981,424.

(60) Provisional application No. 60/798,455, filed on May 5, 2006.

(51) **Int. Cl.**
C07H 21/04 (2006.01)

(52) **U.S. Cl.** **536/23.4**

(58) **Field of Classification Search** None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,867,973	A	9/1989	Goers et al.
5,567,677	A	10/1996	Castensson et al.
5,656,261	A	8/1997	Cerami et al.
5,658,570	A	8/1997	Newman et al.
5,747,035	A	5/1998	Presta et al.
5,843,725	A	12/1998	Sledziewski et al.
5,853,703	A	12/1998	Cerami et al.
5,864,018	A	1/1999	Morser et al.
5,891,341	A	4/1999	Li et al.
6,007,865	A	12/1999	Cerami et al.
6,018,026	A	1/2000	Sledziewski et al.
6,225,448	B1	5/2001	Tao et al.
6,267,958	B1	7/2001	Andya et al.
6,291,646	B1	9/2001	Sledziewski et al.
6,300,099	B1	10/2001	Sledzierwski et al.
6,323,323	B1	11/2001	Sledziewski et al.
6,380,165	B1	4/2002	Al-Abed et al.
6,440,749	B1	8/2002	Cerami et al.
6,465,422	B1	10/2002	Schmidt et al.
6,555,340	B1	4/2003	Schmidt et al.
6,555,651	B2	4/2003	Stern et al.
6,563,015	B1	5/2003	Stern et al.
6,670,136	B2	12/2003	Schmidt et al.
6,677,299	B2	1/2004	Stern et al.
6,753,150	B2	6/2004	Schmidt et al.
6,761,888	B1	7/2004	Schenk
6,790,443	B2	9/2004	Stern et al.
6,825,164	B1	11/2004	Stern et al.
6,939,545	B2	9/2005	Jacobs et al.

6,998,125	B2	2/2006	Hanna et al.
7,026,444	B2	4/2006	Schmidt et al.
7,081,241	B1	7/2006	Schmidt et al.
7,101,838	B2	9/2006	Stern et al.
7,125,675	B2	10/2006	Schmidt et al.
7,189,830	B2	3/2007	Gillies et al.
7,258,857	B2	8/2007	Stern et al.
7,470,521	B2	12/2008	O'Keefe et al.
7,981,424	B2	7/2011	Mjalli et al.
2001/0039256	A1	11/2001	Stern et al.
2001/0053357	A1	12/2001	Stern et al.
2002/0002203	A1	1/2002	Rahbar
2002/0013256	A1	1/2002	Rahbar et al.
2002/0022234	A1	2/2002	Al-Abed et al.
2002/0037496	A1	3/2002	Jacobson et al.
2002/0077293	A1	6/2002	Cahoon et al.
2002/0082273	A1	6/2002	Bush et al.
2002/0086282	A1	7/2002	Pillarsetti et al.
2002/0102604	A1	8/2002	Edwards et al.
2002/0106726	A1	8/2002	Schmidt et al.
2002/0116725	A1	8/2002	Stern et al.
2002/0122799	A1	9/2002	Stern et al.
2003/0144201	A1	7/2003	Tracey et al.
2004/0121372	A1	6/2004	Schmidt et al.
2004/0142391	A1	7/2004	Schmidt et al.
2004/0228855	A1	11/2004	Stern et al.
2005/0008649	A1	1/2005	Shin et al.
2005/0033017	A1	2/2005	Yamamoto et al.
2005/0129682	A1	6/2005	Schmidt et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP 1 380593 1/2004

(Continued)

OTHER PUBLICATIONS

Armour, K. L. et al., "The contrasting IgG-binding interaction of human and herpes simplex virus Fc receptors," *Biochemical Society Transactions*, 2002, vol. 30:495-500.

Bleck, G. T., "An Alternative Method for the Rapid Generation of Stable, High-Expressing Mammalian Cell lines," *BioProcessing Journal*, Sep./Oct. 2005, pp. 1-7.

Canfield, S. M. et al., "The Binding Affinity of Human IgG for Its High Affinity Fc Receptor Is Determined by Multiple Amino Acids in the C_H2 Domain and Is Modulated by the Hinge Region," *J. Exp. Med.*, 1991, vol. 173:1483-1491.

Stevenson, "Characterization of Protein and Peptide Stability and Solubility in Non-Aqueous Solvents", *Current Pharmaceutical Biotechnology*, 2000, vol. 2, No. 2, pp. 165-182.

(Continued)

Primary Examiner — Gregory S Emch

(74) *Attorney, Agent, or Firm* — Kilpatrick Townsend & Stockton LLP

(57) **ABSTRACT**

Disclosed are RAGE fusion proteins comprising RAGE polypeptide sequences linked to a second, non-RAGE polypeptide. The RAGE fusion protein may utilize a RAGE polypeptide domain comprising a RAGE ligand binding site and an interdomain linker directly linked to the N-terminus of an immunoglobulin C_H2 domain. Also disclosed are RAGE fusion protein formulations and the use of the RAGE fusion proteins and RAGE fusion protein formulations as therapeutics for RAGE-mediated pathologies.

5 Claims, 34 Drawing Sheets

U.S. PATENT DOCUMENTS

2005/0170382	A1	8/2005	Stern et al.
2005/0244849	A1	11/2005	Pittman et al.
2006/0025789	A1	2/2006	O'Keefe
2006/0030527	A1	2/2006	Mjalli et al.
2006/0057679	A1	3/2006	O'Keefe et al.
2006/0078562	A1	4/2006	Mjalli et al.
2006/0084145	A1	4/2006	Anderson et al.
2006/0140933	A1	6/2006	Pittman et al.
2007/0014791	A1	1/2007	Schmidt et al.
2007/0099829	A1	5/2007	Stern et al.
2007/0167360	A1	7/2007	Van et al.
2008/0019986	A1	1/2008	Stern et al.
2008/0045455	A1	2/2008	Mjalli
2008/0075733	A1	3/2008	Mjalli et al.
2008/0171701	A1	7/2008	Stern
2008/0199467	A1	8/2008	Mjalli et al.
2008/0207499	A1	8/2008	Barile
2009/0004190	A1	1/2009	Mjalli et al.
2009/0060925	A1	3/2009	Mjalli et al.

FOREIGN PATENT DOCUMENTS

RU	2219947	12/2003
WO	WO 97/26913	7/1997
WO	WO 97/39121	10/1997
WO	WO 97/39125	10/1997
WO	WO 98/22138	5/1998
WO	WO 98/40071	9/1998
WO	WO 99/07402	2/1999
WO	WO 99/13912	3/1999
WO	WO 99/18987	4/1999
WO	WO 94/10308	5/1999
WO	WO 99/45907	9/1999
WO	WO 99/54485	10/1999
WO	WO 00/18970	4/2000
WO	WO 00/20458	4/2000
WO	WO 00/20621	4/2000
WO	WO 01/05422	1/2001
WO	WO 01/12598	2/2001
WO	WO 01/18060	3/2001
WO	WO 01/29269	4/2001
WO	WO 01/76584	10/2001
WO	WO 01/79849	10/2001
WO	WO 01/86002	11/2001
WO	WO 01/92210	12/2001
WO	WO 01/92892	12/2001
WO	WO 02/14519	2/2002
WO	WO 02/30889	4/2002
WO	WO 02/066978	8/2002
WO	WO 02/068636	9/2002
WO	WO 2004/004661	1/2004
WO	WO 2004-016229	2/2004
WO	WO 2004/016229	2/2004
WO	WO 2004/055055	7/2004
WO	WO 2005/019429	3/2005
WO	WO 2005/049852	6/2005
WO	WO 2005/051995	6/2005
WO	WO 2005/061538	7/2005
WO	WO 2005/108584	11/2005
WO	WO 2006/002971	1/2006
WO	WO 2006/012373	2/2006
WO	WO 2006/012415	2/2006
WO	WO 2006-017643	2/2006
WO	WO 2006/017643	2/2006
WO	WO 2006-017647	2/2006
WO	WO 2006/036922	4/2006
WO	WO 2006/119510	11/2006
WO	WO 2007-094926	8/2007
WO	WO 2007/130302	11/2007
WO	WO 2008/157378	6/2008
WO	WO 2008/100470	8/2008

OTHER PUBLICATIONS

Tenno, T. et al., "High-throughput construction method for expression vector of peptides for NMR study suited for isotopic labeling," *Protein Engineering, Design & Selection*, 2004, vol. 17:305-314.

Weiss, P. et al., "Flexible Methodology for Developing Mammalian Cell lines," *The BioPharm International Guide*, Feb. 2006, pp. 30-35.

International Search Report mailed Dec. 15, 2005 corresponding to Application No. PCT/US2005/027694.

Written Opinion of the International Searching Authority mailed Dec. 15, 2005 corresponding to Application No. PCT/US2002/027694.

International Search Report mailed Dec. 11, 2008 corresponding to Application No. PCT/US2008/001786.

Written Opinion of the International Searching Authority mailed Dec. 11, 2008 corresponding to Application No. PCT/US2008/001786.

Nickerson, P. et al., "Prolonged islet allograft acceptance in the absence of interteukin 4 expression", *Transplant Immunology*, Mar. 1996, vol. 4, No. 1, Mar. 1996, pp. 81-65. XP002501957 ISSN: 0966-3274 abstract.

Lekkerkerker, A.N. et al., Potency of HIV-1 envelope glycoprotein gp120 antibodies to inhibit the interaction 01 DC-SIGN with HIV-I gpl20. *Virology. Academic Press. Orlando, US*, vol. 329, No. 2, Nov. 24, 2004 (Nov. 4, 2004), pp. 465-476. XP004613875 ISSN: 0042-6822.

Shoji-Hosaka Emi, et al .. "Enhanced FC-Dependent Cellular Cytotoxicity 01 FC Fusion Proteins Derived From TNF Receptor II and LFA-3 by Fucose Removal From ASN-Linked Oligosaccharides," *Journal 01 Biochemistry, Japanese Biochemical Society / OUP, Tokyo, JP*, vol. 140, No. 6, Jan. 1, 2006, pages 777- 783, XP008079688, ISSN: 0021-924X.

Ausubel, F. et al., *Short Protocols of Molecular Biology*, 4 Edition, Chapter 2.

Arancio, O. et al., RAGE Potentiates A β -induced perturbation of neuronal function in transgenic mice, *EMBO Journal*, Vo. 23, pp. 4096-4105,2004.

Bucciarelli, L. et al., RAGE Blockage Stabilizes established Atherosclerosis in Diabetic Apolipoprotein E-Null Mice, *Circulation* vol. 106, pp. 2827-2835, 2002.

Bonnardel-Phu, E. et al., Acute Modulation of Albumin Microvascular Leakage by Advanced Glycation Ends Products in Microcirculation of Diabetic Rats in Vivo, *Diabetes*, vol. 48, pp. 2052-2058,1999.

Burstein, Y. et al., Partial Amino-Acid Sequence of the Precursor of an Immunoglobulin Light Chain containing NH₂-Terminal PyroglutamicAcid, *Proc. Natl. Acad. Sci. USA*, vol. 73, No. 8, pp. 2604-2608, 1976.

Degenhardt, T. et al., Chemical Modification of Proteins by Methylglyoxal, *Cellular and Molecular Biology*, vol. 44, No. 7, pp. 1139-1145, 1998.

Dyer, D. et al., Accumulation of Maillard Reaction Products in Skin Collagen in Diabetes and Aging, *J. Clin. Invest.*, vol. 91, pp. 2463-2469, 1993.

Dyer, D. et al., Formation of Pentosidine during Nonenzymatic Browning of Proteins by Glucose, *Journal of Biological Chemistry*, vol. 266, No. 18, pp. 11654-11660, 1991.

Flyvbjerg, A. et al., Long Term Renal Effects of a Neutralizing Rage Antibody in Obese Type 2 Diabetic Mice, *Diabetes*, vol. 53, pp. 166-172,2004.

Guo, J. et al., Inflammation-Dependent Cerebral Deposition of Serum Amyloid A Protein in a Mouse Model of Amyloidosis, *Journal of Neuroscience*, vol. 22, No. 14, pp. 5900-5909, 2002.

Hammes, H. et al., Diabetic Retinopathy Risk Correlates with Intracellular Concentrations of the Glycoxidation Product N^ε-(carboxymethyl) Lysine Independently of Glycohaemoglobin Concentrations, *Diabetologia*, vol. 42, DD. 603-607, 1999.

Hofmann, M. et al., RAGE Mediates a Novel Proinflammatory Axis: A Central Cell Surface Receptor for S100/Calgranulin Polypeptides, *Cell*, vol. 97, pp. 889-901, 1999.

Hofmann, M. et al., RAGE and Arthritis: the G82S Polymorphism Amplifies the Inflammatory Response, *Genes and Immunity*. vol. 3, pp. 123-135,2002.

Leder, A. et al., v-Ha-ras Transgene Abrogates the Initiation Step in Mouse Skin Tumorigenesis Effects of Phorbol Esters and Retinoic Acid, *Proc. Natl. Acad. Sci. USA*, vol. 81, pp. 9178-9182, 1990.

Liliensiek, B. et al., Receptor for Advanced Glycation End Products (RAGE) regulates Sepsis but not the Adaptive Immune Response, *Journal of Clinical Investigation*, vol. 113, No. 11, pp. 1641-1650,2004.

- Li, J. et al., Characterization and Functional Analysis of the Promoter of RAGE, the Receptor for Advanced Glycation End Products, *Journal of Biological Chemistry*, vol. 272, No. 26, pp. 16498-16506, 1997.
- Li, J. et al., Sp1-binding Elements in the Promoter of RAGE are Essential for Amphotericin-mediated Gene Expression in Cultured Neuroblastoma Cells, *Journal of Biological Chemistry*, vol. 273, No. 47, pp. 30870-30878, 1998.
- Lugering, N. et al., The Myeloid Related Protein MRP8/14 (27E10 antigen)-Usefulness as a Potential Marker for Disease Activity in Ulcerative Colitis and Putative Biological Function, *European Journal of Clinical Investigation*, vol. 25, pp. 659-664, 1995.
- Luth, H. et al., Age-and-Stage-dependent Accumulation of Advanced Glycation End Products in Intracellular Deposits in Normal and Alzheimer's Disease Brains, *Cerebral Cortex*, vol. 15, pp. 211-220, 2005.
- Miyata, T. et al., 132-Microglobulin Modified with Advanced Glycation End Products is a Major Component of Hemodialysis-associated Amyloidosis, *J. Clin. Invest.*, vol. 92, pp. 1243-1525, 1993.
- Miyata, T. et al., The Receptor for Advanced Glycation End Products (RAGE) is a Central Mediator of the Interaction of AGE-132 Microglobulin with Human Mononuclear Phagocytes via an Oxidant-Sensitive Pathway, *J. Clin. Invest.*, vol. 98, No. 5, pp. 1088-1094, 1996.
- Parkkinen, J. et al., Amphotericin, the 30-kDa Protein in a Family of HMG1-type Polypeptides, *Journal of Biological Chemistry*, vol. 268, No. 26, pp. 19726-19738, 1993.
- Rammes, A. et al., Myeloid-related Protein (MRP) 8 and MRP14, Calcium-binding Proteins of the S100 Family, are Secreted by Activated Monocytes via a Novel, Tubulin-dependent Pathway, *Journal of Biological Chemistry*, vol. 272, No. 14, pp. 9496-9502, 1997.
- Rauvala, H. et al., Isolation and Some Characteristics of an Adhesive Factor of Brain that Enhances Neurite Outgrowth in Central Neurons, *Journal of Biological Chemistry*, vol. 262, No. 34, pp. 16625-16635, 1987.
- Renard, C. et al., The Human and Rat Recombinant Receptors for Advanced Glycation End Products have a High Degree of Homology but Different Pharmacokinetic Properties in Rats, *Journal of Pharmacology and Experimental Therapeutics*, vol. 290, No. 3, pp. 1458-1466, 1999.
- Reddy, S. et al., N^c-(Carboxymethyl)lysine is a Dominant Advanced Glycation End Product (AGE) Antigen in Tissue Proteins, *Biochemistry*, vol. 34, pp. 10872-10878, 1995.
- Rocken, C. et al., Advanced Glycation End Products and Receptor for Advanced Glycation End Products in AA Amyloidosis, *American Journal of Pathology*, vol. 162, No. 4, pp. 1213-1220, 2003.
- Schafer, B. et al., The S100 Family of EF-Hand Calcium-Binding Proteins: Functions and Pathology, *TIBS*, vol. 21, 1996.
- Schleicher, E. et al., Increased Accumulation of the Glycoxidation Product N^ε-(carboxymethyl)lysine in Human Tissues in Diabetes and Aging, *J. Clin. Invest.*, vol. 99, No. 3, pp. 457-468, 1997.
- Schmidt, A. et al., The Dark Side of Glucose, *Nature Medicine*, vol. 1, No. 10, pp. 1002-1004, 1995.
- Simon, R. et al., Peptoids: A Modular Approach to Drug Discovery, *Proc. Natl. Acad. Sci. USA*, vol. 89, pp. 9367-9371, 1992.
- Schmidt, A. et al., The V-Domain of Receptor for Advanced Glycation Endproducts (RAGE) Mediates Binding of AGEs: A Novel Target for Therapy of Diabetic Complications (Abstract), *Circulation*, vol. 96, No. Supp. 194, pp. 137, 1987.
- Taguchi, A. et al., Blockage of RAGE-Amphotericin Signalling Suppresses Tumour Growth and Metastases, *Nature*, vol. 405, pp. 354-360, 2000.
- Tanaka, N. et al., The Receptor for Advanced Glycation End Products is Induced by the Glycation Products themselves and Tumor Necrosis Factor- α through Nuclear Factor- κ B, and by 17 β -Estradiol through Sp-1 in Human Vascular Endothelial Cells, *Journal of Biological Chemistry*, vol. 275, Issue 18, pp. 25781-25790, 2000.
- Teillet, L. et al., Food Restriction prevents Advanced Glycation End Product Accumulation and Retards Kidney Aging in Lean Rats, *J. Am. Soc. Nephrol.*, vol. 11, pp. 1488-1497, 2000.
- Vlassara, H. et al., Advanced Glycation End-products and Atherosclerosis, *The Finnish Medical Society DUODECIM, Ann. Med.*, vol. 28, pp. 419-426, 1996.
- Wilbur, W. et al., Rapid Similarity Searches of Nucleic Acid and Protein Data Banks, *Proc. Natl. Acad. Sci., USA*, vol. 80, pp. 726-730, 1983.
- Yan, S. et al., Receptor-dependent Cell Stress and Amyloid Accumulation in Systemic Amyloidosis, *Nature Medicine*, vol. 6, No. 6, pp. 643-651, 2000.
- Yan, S. et al., RAGE and Amyloid-13 Peptide Neurotoxicity in Alzheimer's Disease, *Nature*, vol. 382, pp. 685-691, 1996.
- Yeh, C. et al., Requirement for p38 and p44/p42 Mitogen-Activated Protein Kinases in RAGE-Mediated Nuclear Factor- κ B Transcriptional Activation and Cytokine Secretion, *Diabetes*, vol. 50, pp. 1495-1504, 2001.
- Zhou, Z. et al., Receptor for AGE (RAGE) Mediates Neointimal Formation in Response to Arterial Injury, *Circulation*, vol. 107, pp. 2238-2243, 2003.
- Zhou, Z. et al., Regulation of Osteoclast Function and Bone Mass by RAGE, *Journal of Experimental Medicine*, vol. 203, No. 4, pp. 1067-1080, 2006.
- Zimmer, D. et al., The S100 Protein Family: History, Function, and Expression, *Brain Research Bulletin*, vol. 37, No. 4, pp. 417-429, 1995.
- Crall, F. et al., The Extramural and Intramural Coronary Arteries in Juvenile Diabetes Mellitus, *American Journal of Medicine*, vol. 64, pp. 221-230, 1978.
- Hamby, R. et al., Reappraisal of the Role of the Diabetic State in Coronary Artery Disease, *Chest*, vol. 2, pp. 251-257, 1976.
- Johnson, M. et al., Antioxidant with Marked Lipid and Glucose-Lowering Activity in Diabetic Rats and Mice, *Diabetes*, vol. 42, pp. 1179-1186, 1993.
- Kannel, W. et al., Diabetes and Glucose Tolerance as Risk Factors for Cardiovascular Disease: The Framingham Study, *Diabetic Care*, vol. 2, pp. 120-126, 1979.
- Kannel, W. et al., Diabetes and Cardiovascular Disease-The Framingham Study, *J. Am. Med. Assoc.*, vol. 241, pp. 2035-2038, 1979.
- Needleman, S. et al., A General Method Applicable to the Search for Similarities in the Amino Acid Sequence of Two Proteins, *J. Mol. Biol.*, vol. 48, pp. 443-453, 1970.
- Park, L. et al., Suppression of Accelerated Diabetic Atherosclerosis by the Soluble Receptor for Advanced Glycation Endproducts, *Nature Med.*, vol. 4, pp. 1025-1031, 1998.
- Pearson, W. et al., Improved Tools for Biological Sequence Comparison, *Proc. Natl. Acad. Sci. USA*, vol. 85, pp. 2444-2448, 1998.
- Pyorala, K. et al., Diabetes and Atherosclerosis, An Epidemiologic View, *Diabetes/Metabolism Reviews*, vol. 3, No. 2, pp. 463-524, 1987.
- Smith, T. et al., Comparison of Biosequences, *Advances in Applied Mathematics*, Chapter 2, pp. 428-488, 1981.
- Sousa, M. et al., Interaction of the Receptor for Advanced Glycation End Products (RAGE) with Transthyretin Triggers Nuclear Transcription Factor κ B (NF- κ B) Activation, *Laboratory Investigation*, vol. 80, No. 7, pp. 1101-1110, 2000.
- Waller, B. et al., Status of the Coronary Arteries at Necropsy in Diabetes Mellitus with Onset after Age 30 years, *Am. J. Med.*, vol. 69, pp. 498-506, 1980.
- Yan, S. et al., Receptor-dependent Cell Stress and Amyloid Accumulation in Systemic Amyloidosis, *Nature Medicine*, vol. 6, pp. 643-651, 2000.
- Petzold, A. et al., Cerebrospinal Fluid S100B correlates with Brain Atrophy in Alzheimer's Disease, *Neuroscience Letters*, vol. 336, pp. 167-170, 2003.
- Yan, S. et al., Suppression of Experimental Autoimmune Encephalomyelitis by Selective Blockade of Encephalitogenic T-cell Infiltration of the Central Nervous System, *Nature Medicine*, vol. 9, No. 3, pp. 287-293, 2003.
- Lue, I. et al., Involvement of Microglial Receptor for Advanced Glycation Endproducts (RAGE) in Alzheimer's Disease: Identification of a Cellular Activation Mechanism, *Experimental Neurology*, vol. 171, pp. 29-45, 2001.
- Ellison, J. et al., Linkage and Sequence Homology of Two Human Immunoglobulin V Heavy Chain Constant Region Genes, *Proc. Natl. Acad. Sci. USA*, vol. 79, pp. 1984-1988, 1982.

- Sasaki, N. et al., Immunohistochemical Distribution of the Receptor for Advanced Glycation End Products in Neurons and Astrocytes in Alzheimer's Disease, *Brain Research*, vol. 888, pp. 256-262, 2001.
- Chavakis, T. et al., RAGE (Receptor for Advanced Glycation End Products): A Central Player in the Inflammatory Response, *Microbes and Infection*, vol. 6, pp. 1219-1225, 2004.
- Jones, A. et al., Analysis of Polypeptides and Proteins, *Advances Drug Delivery Reviews*, vol. 10, pp. 29-90, 1993.
- Bucciarelli, I. et al., RAGE is a Multiligand Receptor of the Immunoglobulin Superfamily, Implication for Homeostasis and Chronic Disease, *Cell. Mol. life Sci.*, vol. 59, pp. 1117-1128, 2002.
- Kokkola, R. et al., RAGE is the Major Receptor for the Proinflammatory Activity of HMGB1 in Rodent Macrophages, *Scandinavian Journal of Immunology*, vol. 61, pp. 1-9, 2005.
- Yan, S. et al., RAGE-A13 Interactions in the Pathophysiology of Alzheimer Disease, *Restorative Neurology and Neuroscience*, vol. 12, pp. 167-173, 1998.
- Hudson, B. et al., Blockade of Receptor for Advanced Glycation Endproducts: A New Target for Therapeutic Intervention in Diabetic Complications and Inflammatory Disorders, *Archives of Biochemistry and Biophysics*, vol. 419, pp. 80-88, 2003.
- Morgan, B. et al., Chapter 26—Approaches to the Discovery of Non-Peptide Ligands for Peptide Receptors and Peptidases, *Ann Reports Med. Chem.*, vol. 24, pp. 243-252, 1989.
- Robertson, W. et al., Atherosclerosis in Persons with Hypertension and Diabetes Mellitus, *Laboratory Investigation*, vol. 18, No. 5, pp. 538-551, 1968.
- Lee et al., *Peptide and Protein Drug Delivery*, Marcel Dekker Inc., pp. 247-301, 1991.
- Patent Cooperation Treaty, International Search Report, International Application No. PCT/US2007/001686, mailed Aug. 29, 2007.
- Mohler, K. et al., Soluble Tumor Necrosis Factor (TNF) Receptors are Effective Therapeutic Agents in Lethal Endotoxemia and Function Simultaneously as Both TNF Carriers and TNF Antagonists. *Journal of Immunology*, vol. 151, No. 3, pp. 1548-1561, 1993.
- Schmidt, Ann Marie et al., "Isolation and Characterization of Two Binding Proteins for Advanced Glycosylation End Products from Bovine Lung Which Are Present on the Endothelial Cell Surface," *The Journal of Biological Chemistry*, pp. 14987-14997, vol. 267, No. 21. The American Society for Biochemistry and Molecular Biology, Inc., Jul. 25, 1992).
- Wines, Bruce D. et al., "The IgG Fc Contains Distinct Fc Receptor (FcR) Binding Sites: The Leukocyte Receptors FcγRI and FcγRIIa Bind to a Region in the Fc Distinct from That Recognized by Neonatal FcR and Protein A1," *The Journal of Immunology*, 164: 5313-5318. The American Association of Immunologists, (2000).
- Renard, C. et al., "Recombinant Advanced Glycation End Product Receptor Pharmacokinetics in Normal and Diabetic Rats," *Molecular Pharmacology*, 52: 54-62, The American Society for Pharmacology and Experimental Therapeutics. (1997).
- Van, S. D. et al., "Amyloid-β Peptide-Receptor for Advanced Glycation Endproduct Interaction Elicits Neuronal Expression of Macrophage-Colony Stimulating Factor: A Proinflammatory Pathway in Alzheimer Disease," *Proc. Natl. Acad. Sci.*, 94: pp. 5296-5301, 1997.
- Hori, et al., "The Receptor for Advanced Glycation End Products (RAGE) is a Cellular Binding Site for Amphotericin," *The Journal of Biological Chemistry*, 270(43); pp. 25752-25761, (1995).
- Huttunen et al., Receptor for Advanced Glycation End Products-Binding COOH-Terminal Motif of Amphotericin Inhibits Invasive Migration and Metastasis, *Cancer Research*, 62(12); 4805-4811, (2002).
- International Search Report issued Oct. 26, 2005 in connection with PCT Publication No. WO 03/025996.
- Kunzendorf, U. et al., "Immunomodulation in Experimental and Clinical Nephrology Using Chimeric Proteins," *Kidney Blood Press Res.* 19(3-4):201-4, Department of Internal Medicine and Nephrology, Universitätsklinikum Benjamin Franklin, (1996).
- Li, J., et al., "Spl-Binding Elements in the Promoter of RAGE are Essential for Amphotericin-mediated Gene Expression in Cultured Neuroblastoma Cells," *The Journal of Biological Chemistry*, 273(47); pp. 30870-30878, (1981).
- Neeper, Michael et al., "Cloning and Expression of a Cell Surface Receptor for Advanced Glycosylation End Products of Proteins," *The Journal of Biological Chemistry*, pp. 14998-15004, vol. 267, No. 21, The American Society for Biochemistry and Molecular Biology, Inc., Jul. 25, 1992.
- Mickle et al., "Genotype-Phenotype Relationships in Cystic Fibrosis," *Med. Clin. North Am.*, 84(2); pp. 597-607, May 2000.
- "Recombinant Human RAGE/Fe Chimera," R&D Systems, Inc., Catalog No. 1145-RG, Mar. 5, 2004.
- Schmidt, A. et al., "Receptor for Advanced Glycation End Products (AGEs) has a Central Role in Vessel Wall Interactions and Gene Activation in Response to Circulating AGE Proteins," *Proc. Natl. Acad. Sci.* 91; pp. 8807-8811, (1994).
- Schmidt, et al., "The Multiligand Receptor RAGE as a Progression Factor Amplifying Immune and Inflammatory Responses," *J. Clin. Invest.* 949-955, vol. 108(7), Oct. 2001.
- Stern et al., "Receptor for Advanced Glycation Endproducts (RAGE) and the Complications of Diabetes," *Ageing Research Reviews*, 1(1); 1-15, (2002).
- Tanaka, et al., "The Receptor for Advanced Glycation End Products is Induced by the Glycation Products Themselves and Tumor Necrosis Factor-α through Nuclear Factor-κB, and by 17β-Estradiol through Sp-1 in Human Vascular Endothelial Cells," *The Journal of Biological Chemistry*, 275(33); pp. 25781-25790 (2000).
- Wautier, et al., "Receptor-Mediated Endothelial Cell Dysfunction in Diabetic Vasculopathy. Soluble Receptor for Advanced Glycation End Products Blocks Hyperpermeability in Diabetic Rats," *J. Clin. Invest.*, 97(1); pp. 238-243, (1996).
- Yan et al "Two-Amino Acid Molecular Switch in an Epithelial Morphogen that Regulates Binding to Two Distinct Receptors," *Science* 290; 523-527, (2000).
- Morton, Phillip A et al., "Differential Effects of CTLA-4 Substitutions on the Binding of Human CD80 (B7-1) and CD86 (B7-2)," *The Journal of Immunology*, 156: 1047-1054, The American Association of Immunologists, 1996.
- Peppel et al., "A Tumor Necrosis Factor (TNF) Receptor-igG Heavy Chain Chimeric Protein as a Bivalent Antagonist of TNF Activity," *J. Exp. Med.* 1;174 (6): 1483-9, Dec. 2000.
- Rouhiainen et al., "Regulation of Monocyte Migration by Amphotericin (HMGB1)" *Blood*, vol. 104, pp. 1174-1176, (2004).
- Chelius et al., "Formation of Pyroglutamic Acid from N-Terminal Glutamic Acid in Immunoglobulin Gamma Antibodies" *Analytical Chemistry*, vol. 78, pp. 2370-2376, (2006).
- Written Opinion of the International Search Authority for related PCT Application, PCT/US2007/010125, mailed Feb. 22, 2008.
- International Search Report for related PCT Application, PCT/US2007/010125, mailed Feb. 22, 2008.

FIG. 1A**SEQ ID NO: 1**

MAAGTAVGAW VLVLSLWGAV VGAQNITARI GEPLVLKCKG APKKPPQRLE
 WKLNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ
 AMNRNGKETK SNYRVRVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY
 PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
 DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
 PGGTVTLTCE VPAQPSPQIH WMKDG VPLPL PPSPVLILPE IGPQDQGTYS
 CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGG SGLGT LALALGILGG
 LGTAALLIGV ILWQRRQRRG EERKAPENQE EEEERAELNQ SEEPEAGESS
 TGGP

SEQ ID NO: 2

WKLNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ
 AMNRNGKETK SNYRVRVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY
 PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
 DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
 PGGTVTLTCE VPAQPSPQIH WMKDG VPLPL PPSPVLILPE IGPQDQGTYS
 CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGG SGLGT LALALGILGG
 LGTAALLIGV ILWQRRQRRG EERKAPENQE EEEERAELNQ SEEPEAGESS
 TGGP

FIG. 1B**SEQ ID NO: 3**

WKLNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ
 AMNRNGKETK SNYRVRVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY
 PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
 DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
 PGGTVTLTCE VPAQPSPQIH WMKDG VPLPL PPSPVLILPE IGPQDQGTYS
 CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGG SGLGT LALALGILGG
 LGTAALLIGV ILWQRRQRRG EERKAPENQE EEEERAELNQ SEEPEAGESS
 TGGP

FIG. 1C**SEQ ID NO: 4**

MAAGTAVGAW VLVLSLWGAV VGAQNITARI GEPLVLKCKG APKKPPQRLE
WKLNTGRTEA WKVLSPOGGG PWDSVARVLP NGSFLPAVG IQDEGIFRCQ
AMNRNGKETK SNYRVRVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY
PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
PGGTVTLTCE VPAQPSPQIH WMKDGVPPLP PPSVLILPE IGPQDQGTYS
CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGGSGLG

SEQ ID NO: 5

WKLNTGRTEA WKVLSPOGGG AQNITARI GEPLVLKCKG APKKPPQRLE
AMNRNGKETK SNYRVRVYQI PWDSVARVLP NGSFLPAVG IQDEGIFRCQ
PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH SELTAGVPNK VGTCVSEGSY
DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
PGGTVTLTCE VPAQPSPQIH WMKDGVPPLP PPSVLILPE IGPQDQGTYS
CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGGSGLG

SEQ ID NO: 6

WKLNTGRTEA WKVLSPOGGG QNITARI GEPLVLKCKG APKKPPQRLE
AMNRNGKETK SNYRVRVYQI PWDSVARVLP NGSFLPAVG IQDEGIFRCQ
PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH SELTAGVPNK VGTCVSEGSY
DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
PGGTVTLTCE VPAQPSPQIH WMKDGVPPLP PPSVLILPE IGPQDQGTYS
CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGGSGLG

FIG. 1D**SEQ ID NO: 7**

AQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVR

SEQ ID NO: 8

QNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVR

SEQ ID NO: 9

AQNITARI GEPLVLKCKG APKKPPQRLE WK

SEQ ID NO: 10

QNITARI GEPLVLKCKG APKKPPQRLE WK

SEQ ID NO: 11

PEIVDSA SELTAGVPNK VGTCVSEGSY
PAGTLSWHL D GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
DPRPTFSCSF SPGLPRHRAL R

SEQ ID NO: 12

PRVW EPVPLEEVQL VVEPEGGAVA
PGGTVTLTCE VPAQPSPQIH WMKDGVPPLP PPSVLILPE IGPQDQGTYS
CVATHSSHGP QESRAVS

SEQ ID NO: 13

AQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGK

FIG. 1 E**SEQ ID NO: 14**

QNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSFLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGK

SEQ ID NO: 15

AQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSFLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAG

SEQ ID NO: 16

QNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSFLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAG

SEQ ID NO: 17

AQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSFLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQ

SEQ ID NO: 18

QNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSFLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQ

FIG. 1F**SEQ ID NO: 19**

AQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA P

SEQ ID NO: 20

QNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA P

SEQ ID NO: 21

VYQIPGK

SEQ ID NO: 22

TAPIQPRVWE PVPLEEVQLV VEPEGGAVAP

SEQ ID NO: 23

VYQIPGKPEI VDSASELTAG

SEQ ID NO: 24

TAPIQ

FIG. 1G**SEQ ID NO: 25**

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACCTACC GAGTCCGTGT
CTAC

SEQ ID NO: 26

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACCTACC GAGTCCGTGT
CTACCAGATT CCTGGGAAG

SEQ ID NO: 27

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACCTACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGT

FIG. 1H**SEQ ID NO:28**

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCTGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACCTACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGTGT TCCCAATAAG GTGGGGACAT GTGTGTCAGA GGGGAGCTAC
CCTGCAGGGA CTCTTAGCTG GCACTTGGAT GGAAGCCCC TGGTGCCTAA
TGAGAAGGGA GTATCTGTGA AGGAACAGAC CAGGAGACAC CCTGAGACAG
GGCTCTTCAC ACTGCAGTCG GAGCTAATGG TGACCCAGC CCGGGGAGGA
GATCCCCGTC CCACCTTCTC CTGTAGCTTC AGCCAGGCC TTCCCCGACA
CCGGGCCTTG CGCACAGCCC CCATCCAGCC CCGTGTCTGG

SEQ ID NO:29

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCTGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACCTACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGTGT TCCCAATAAG GTGGGGACAT GTGTGTCAGA GGGGAGCTAC
CCTGCAGGGA CTCTTAGCTG GCACTTGGAT GGAAGCCCC TGGTGCCTAA
TGAGAAGGGA GTATCTGTGA AGGAACAGAC CAGGAGACAC CCTGAGACAG
GGCTCTTCAC ACTGCAGTCG GAGCTAATGG TGACCCAGC CCGGGGAGGA
GATCCCCGTC CCACCTTCTC CTGTAGCTTC AGCCAGGCC TTCCCCGACA
CCGGGCCTTG CGCACAGCCC CCATCCAGCC CCGTGTCTGG GAGCCTGTGC
CTCTGGAGGA GGTCCAATTG GTGGTGGAGC CAGAAGGTGG AGCAGTAGCT
CCT

FIG. 11**SEQ ID NO: 38**

PSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG
VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVS NKALPAP
IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW
ESNGQPENNY KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA
LHNHYTQKSL SLSPGK

SEQ ID NO: 39

CCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT
CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG
ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT
CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCATCGA GAAAACCATC
TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC
ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA
AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT CCGACGGCTC
CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATGA

SEQ ID NO: 40

PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW
YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA
LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI
AVEWESNGQP ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSV
MHEALHNHYT QKSLSLSPGK

FIG. 1J**SEQ ID NO: 41**

CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT TCCTCTTCCC
CCCAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT
GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA
GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC
AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
CTCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG
AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGATGAG CTGACCAAGA
ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAAC TACAAGACCAC
GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA
CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG
ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC
TCCGGGTAAA TGA

SEQ ID NO: 42

PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW
YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA
LPAPIEKTIS KAK

SEQ ID NO: 43

 GQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI
AVEWESNGQP ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV
MHEALHNHYT QKSLSLSPGK

SEQ ID NO: 44

ISI IEPGEEGPTA GSVGGSGGLGT LA

FIG. 1K**SEQ ID NO: 45**

pENITARI GEPLVLKCKG APKKPPQRLE
WKLNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ
AMNRNGKETK SNYRVRVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY
PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
PGGTVTLTCE VPAQPSPQIH WMKDGVPLPL PPSPVLILPE IGPQDQGTYS
CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGGSGLG

SEQ ID NO: 46

pENITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVR

SEQ ID NO: 47

pENITARI GEPLVLKCKG APKKPPQRLE WK

SEQ ID NO: 48

pENITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGK

FIG. 1L**SEQ ID NO: 49**

pENITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAG

SEQ ID NO: 50

pENITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQ

SEQ ID NO: 51

pENITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPOGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA P

FIG. 1M**SEQ ID NO: 52**

CCGTCAG TCTTCCTCTT CCCCCAAAA CCAAGGACA CCCTCATGAT
CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG
ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT
CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCATCGA GAAAACCATC
TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC
ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA
AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT CCGACGGCTC
CTTCTTCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
ACGCAGAAGA GCCTCTCCCT GTCTCCCGGG AAATGA

SEQ ID NO: 53

CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT TCCTCTTCCC
CCCAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTACAT
GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA
GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC
AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG
AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGATGAG CTGACCAAGA
ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACACT ACAAGACCAC
GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCTCTAC AGCAAGCTCA
CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG
ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC
TCCCGGGAAA TGA

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAAC TACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGTGT TCCCAATAAG GTGGGGACAT GTGTGTCAGA GGGGAGCTAC
CCTGCAGGGA CTCTTAGCTG GCACTTGGAT GGAAGCCCC TGGTGCCTAA
TGAGAAGGGA GTATCTGTGA AGGAACAGAC CAGGAGACAC CCTGAGACAG
GGCTCTTCAC ACTGCAGTCG GAGCTAATGG TGACCC CAGC CCGGGGAGGA
GATCCCCGTC CCACCTTCTC CTGTAGCTTC AGCC CAGGCC TTCCCCGACA
CCGGGCCTTG CGCACAGCCC CCATCCAGCC CCGTGTCTGG GAGCCTGTGC
CTCTGGAGGA GGTCCAATTG GTGGTGGAGC CAGAAGGTGG AGCAGTAGCT
CCTCCGTCAG TCTTCCTCTT CCCCCAAAA CCAAGGACA CCCTCATGAT
CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG
ACCCTGAGGT CAAGTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT
CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCATCGA GAAAACCATC
TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC
ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA
AAGGCTTCTA TCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT CCGACGGCTC
CTTCTTCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATGA

(SEQ ID NO: 30)

FIG. 2A

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCAAGA AACCACCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCA ACTACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGTGT TCCCAATAAG GTGGGGACAT GTGTGTCAGA GGGGAGCTAC
CCTGCAGGGA CTCTTAGCTG GCACTTGGAT GGAAGCCCC TGGTGCCTAA
TGAGAAGGGA GTATCTGTGA AGGAACAGAC CAGGAGACAC CCTGAGACAG
GGCTCTTCAC ACTGCAGTCG GAGCTAATGG TGACCCAGC CCGGGGAGGA
GATCCCCGTC CCACCTTCTC CTGTAGCTTC AGCCAGGCC TTCCCCGACA
CCGGGCCTTG CGCACAGCCC CCATCCAGCC CCGTGTCTGG GAGCCTGTGC
CTCTGGAGGA GGTCCAATTG GTGGTGGAGC CAGAAGGTGG AGCAGTAGCT
CCTCCGTCAG TCTTCCTCTT CCCCCAAA CCAAGGACA CCCTCATGAT
CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG
ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT
CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCATCGA GAAAACCATC
TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC
ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA
AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
CCGAGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT CCGACGGCTC
CTTCTTCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
ACGCAGAAGA GCCTCTCCCT GTCTCCCGGG AAATGA

(SEQ ID NO: 54)

FIG. 2B

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCAAGA AACCACCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACCTACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGTCC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC
ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA
CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC
ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT
GTGGTCAGCG TCCTCACCCT CCTGCACCAG GACTGGCTGA ATGGCAAGGA
GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA
CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACACAGGT GTACACCCTG
CCCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC TGACCTGCCT
GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG
GGCAGCCGGA GAACAACCTAC AAGACCACGC CTCCCCTGCT GGACTCCGAC
GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA
GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC
ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG A

(SEQ ID NO: 31)

FIG. 3A

ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG
GGGGGCAGTA GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC
TGGTGCTGAA GTGTAAGGGG GCCCCCAAGA AACCACCCCA GCGGCTGGAA
TGGAAACTGA ACACAGGCCG GACAGAAGCT TGGAAGGTCC TGTCTCCCA
GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCTTCCC AACGGCTCCC
TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAAC TACC GAGTCCGTGT
CTACCAGATT CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA
CGGCTGGTCC GTCAGTCTTC CTCTTCCCC CAAAACCCAA GGACACCCTC
ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA
CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC
ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT
GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA
GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCC ATCGAGAAAA
CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG
CCCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC TGACCTGCCT
GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG
GGCAGCCGGA GAACA ACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC
GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA
GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC
ACTACACGCA GAAGAGCCTC TCCCTGTCTC CCGGGAAATG A

(SEQ ID NO: 55).

FIG. 3B

SEQ ID NO: 32

MAAGTAVGAW VLVLSLWGAV VGAQNITARI GEPLVLKCKG APKKPPQRLE
WKLNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ
AMNRNGKETK SNYRVRVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY
PAGTLSWHLG GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG
DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA
PPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ
PENNYKTTPP VLDSGDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
TQKSLSLSPG K

SEQ ID NO: 33

AQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLG GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA PPSVFLFPPK PKDTLMISRT
PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSGDGSFFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K

SEQ ID NO: 34

QNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLG GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA PPSVFLFPPK PKDTLMISRT
PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSGDGSFFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K

SEQ ID NO: 56

pENITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA WKVLSPQGGG
PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLG GKPLVPNEKG
VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL
RTAPIQPRVW EPVPLEEVQL VVEPEGGAVA PPSVFLFPPK PKDTLMISRT
PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSGDGSFFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K

FIG. 4

SEQ ID NO: 35

MAAGTAVGAW	VLVLSLWGAV	VGAQNITARI	GEPLVLKCKG	APKKPPQRLE
WKLNTGRTEA	WKVLSPOGGG	PWDSVARVLP	NGSLFLPAVG	IQDEGIFRCQ
AMNRNGKETK	SNYRVRVYQI	PGKPEIVDSA	SELTAGPSVF	LFPPKPKDTL
MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR
VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL
PPSRDELTKN	QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY	KTTPPVLDSD
GSFFLYSKLT	VDKSRWQQGN	VFSCSVMHEA	LHNHYTQKSL	SLSPGK

SEQ ID NO: 36

AQNITARI	GEPLVLKCKG	APKKPPQRLE	WKLNTGRTEA	WKVLSPOGGG
PWDSVARVLP	NGSLFLPAVG	IQDEGIFRCQ	AMNRNGKETK	SNYRVRVYQI
PGKPEIVDSA	SELTAGPSVF	LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP
EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC
KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG
FYPSDIAVEW	ESNGQPENNY	KTTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN
VFSCSVMHEA	LHNHYTQKSL	SLSPGK		

SEQ ID NO: 37

QNITARI	GEPLVLKCKG	APKKPPQRLE	WKLNTGRTEA	WKVLSPOGGG
PWDSVARVLP	NGSLFLPAVG	IQDEGIFRCQ	AMNRNGKETK	SNYRVRVYQI
PGKPEIVDSA	SELTAGPSVF	LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP
EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC
KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG
FYPSDIAVEW	ESNGQPENNY	KTTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN
VFSCSVMHEA	LHNHYTQKSL	SLSPGK		

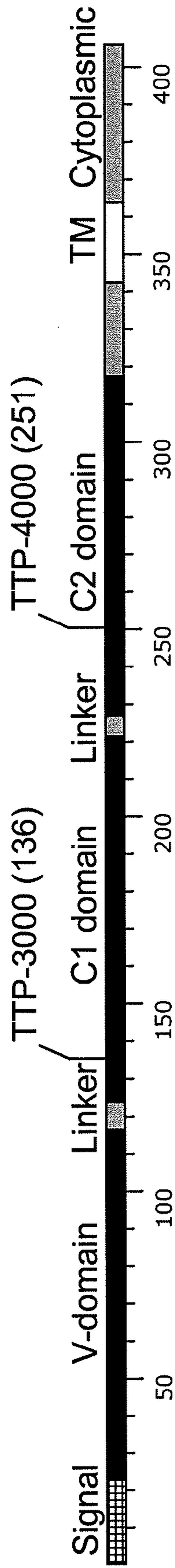
SEQ ID NO: 57

pENITARI	GEPLVLKCKG	APKKPPQRLE	WKLNTGRTEA	WKVLSPOGGG
PWDSVARVLP	NGSLFLPAVG	IQDEGIFRCQ	AMNRNGKETK	SNYRVRVYQI
PGKPEIVDSA	SELTAGPSVF	LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP
EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC
KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG
FYPSDIAVEW	ESNGQPENNY	KTTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN
VFSCSVMHEA	LHNHYTQKSL	SLSPGK		

FIG. 5

FIG. 6A

hRAGE Domain Structure



HUMAN Ig gamma-1 Fc

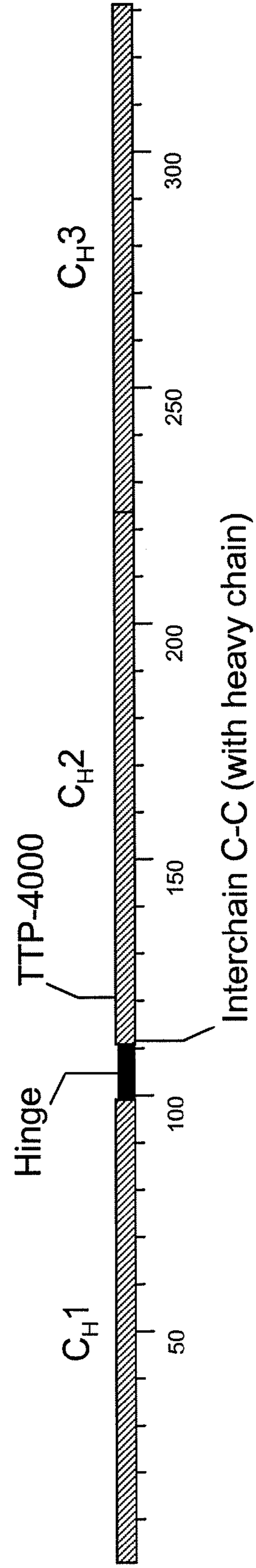
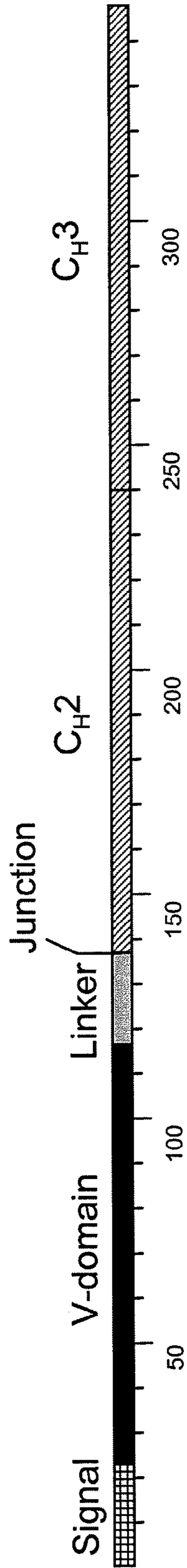


FIG. 6B

TTP-3000



TTP-4000

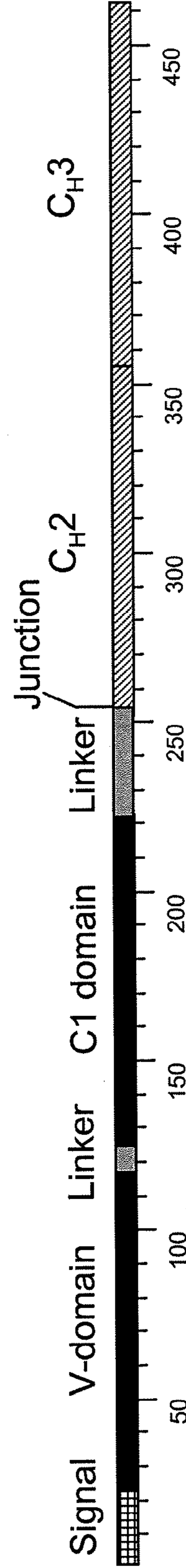


FIG. 7



FIG. 8

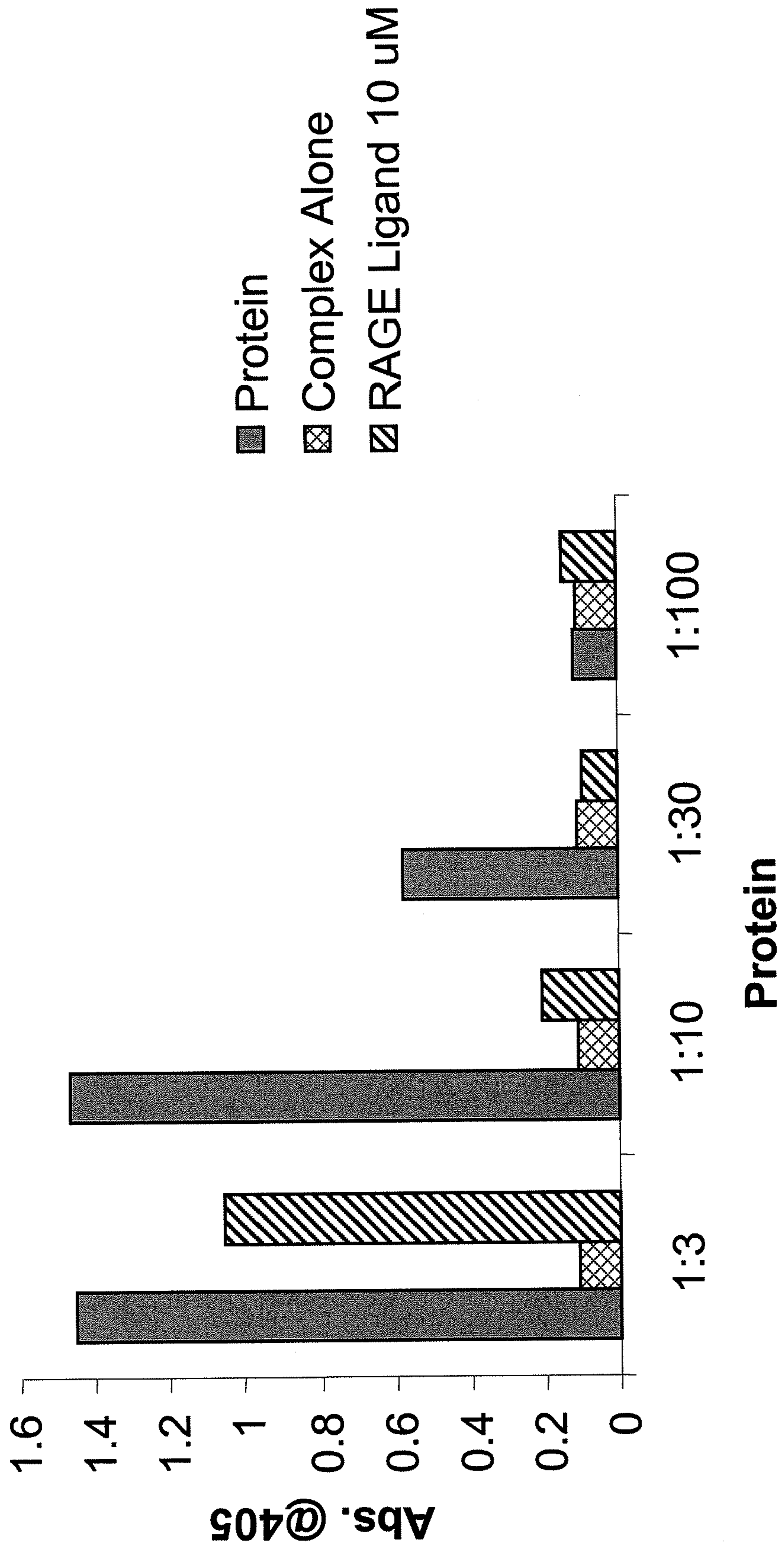


FIG. 9

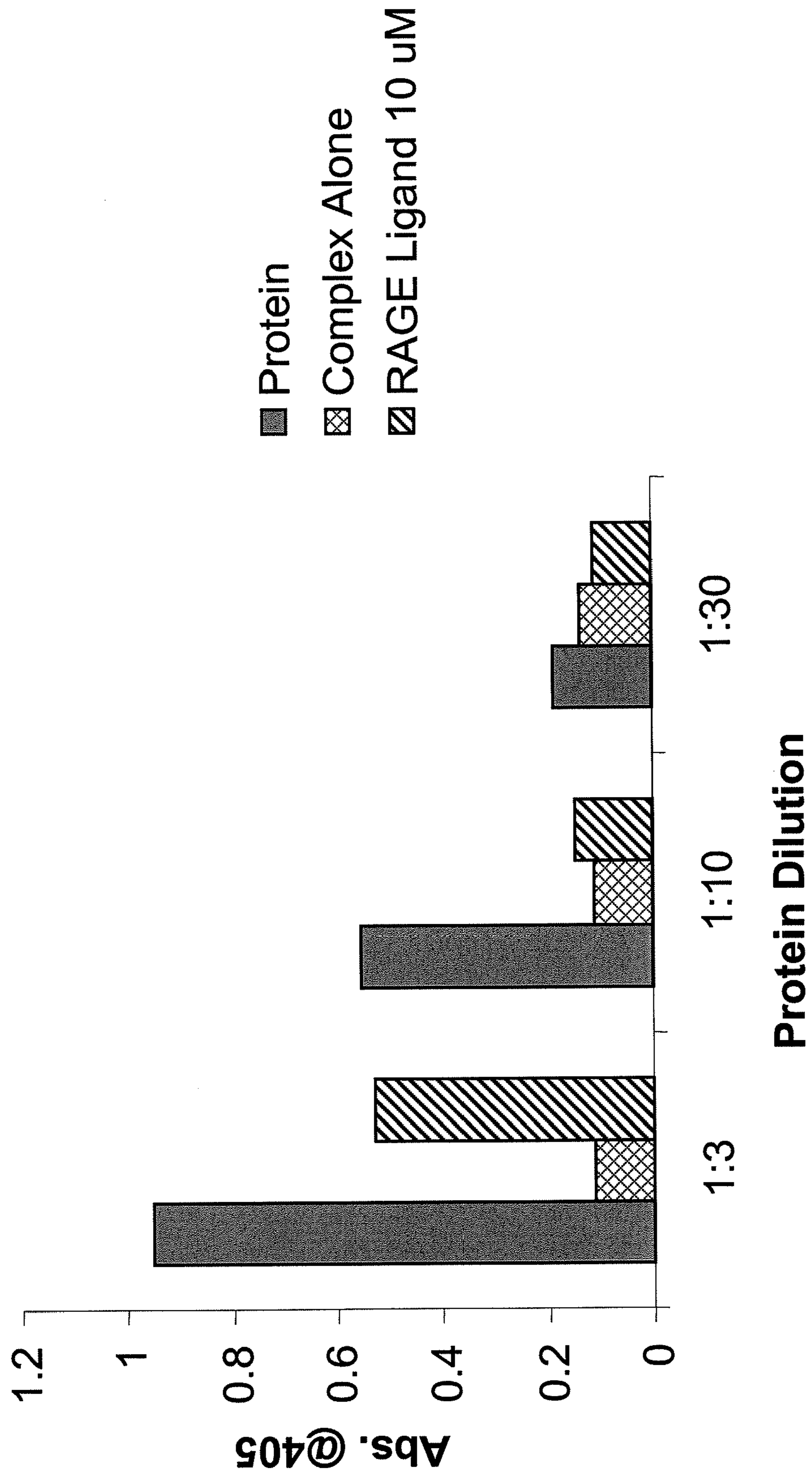


FIG. 10

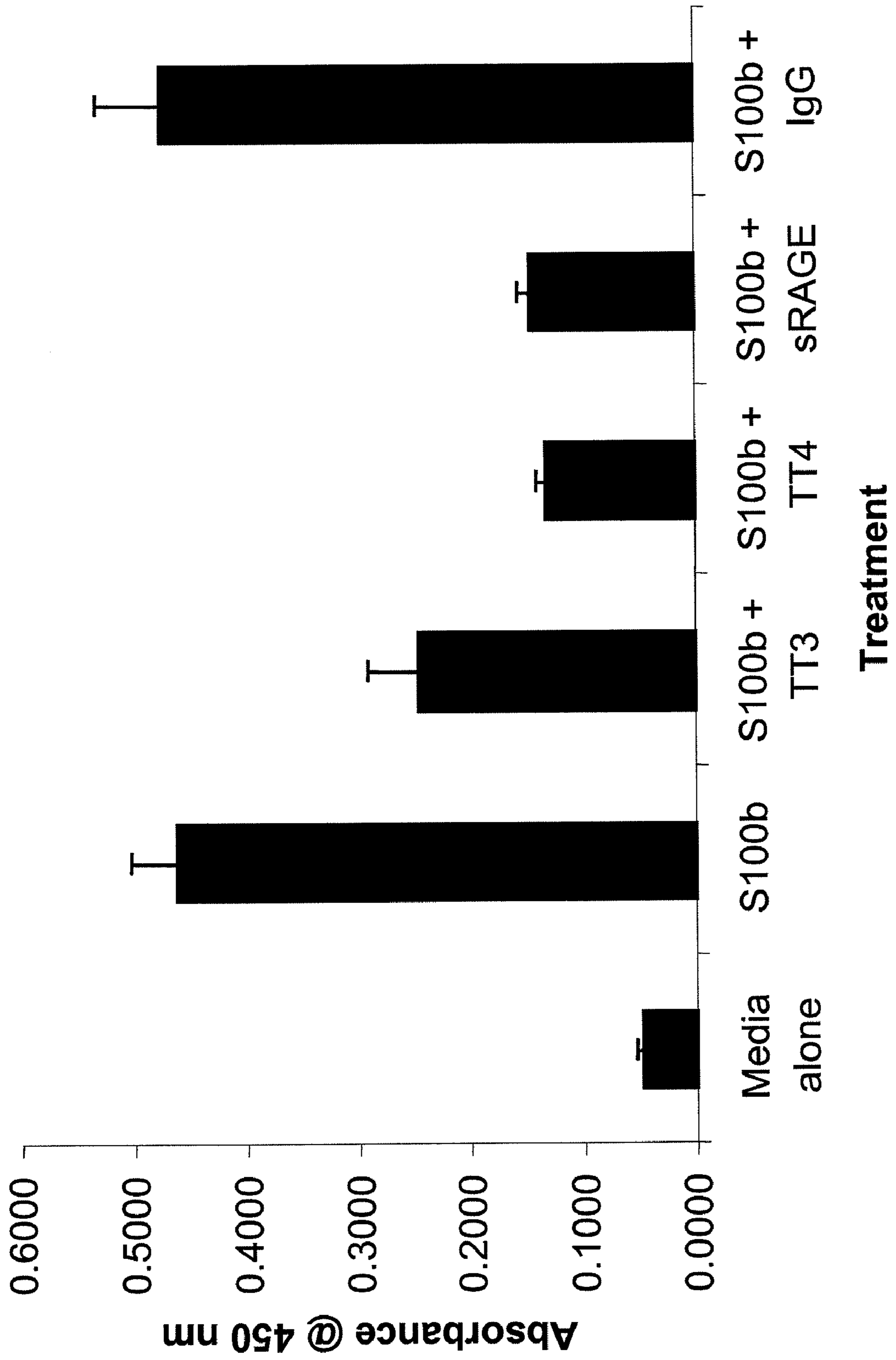


FIG. 11

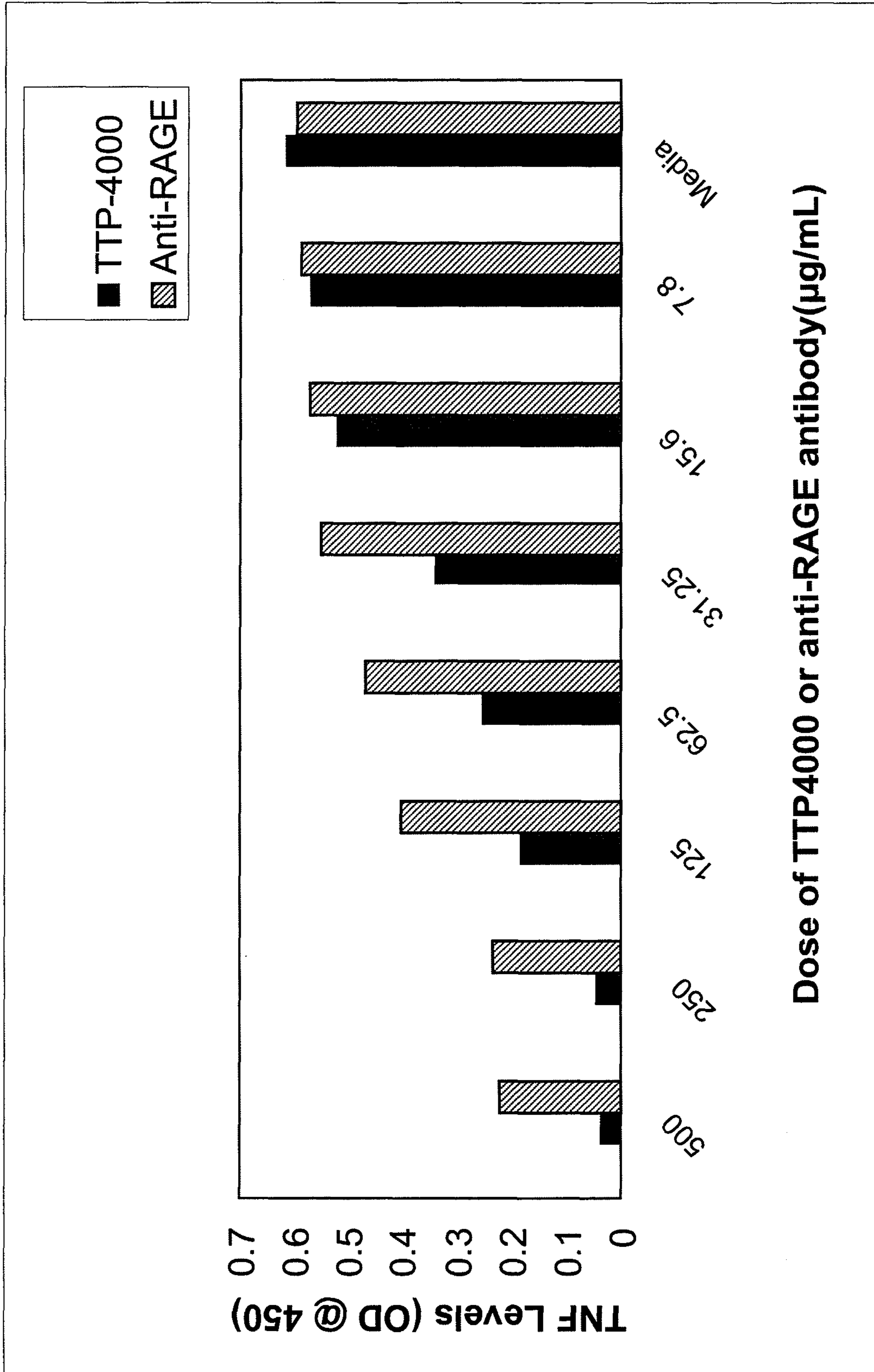


FIG. 12

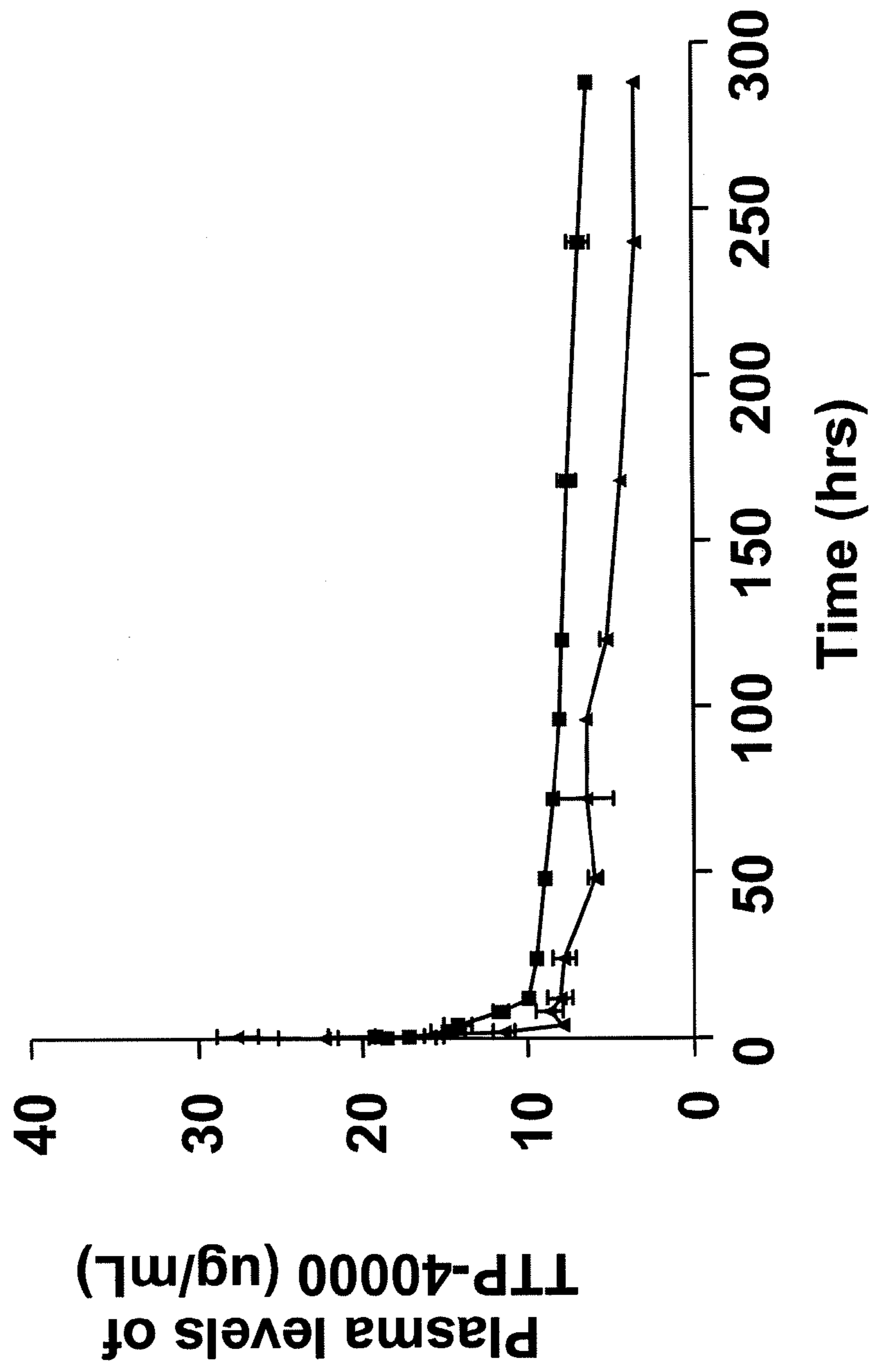


FIG. 13

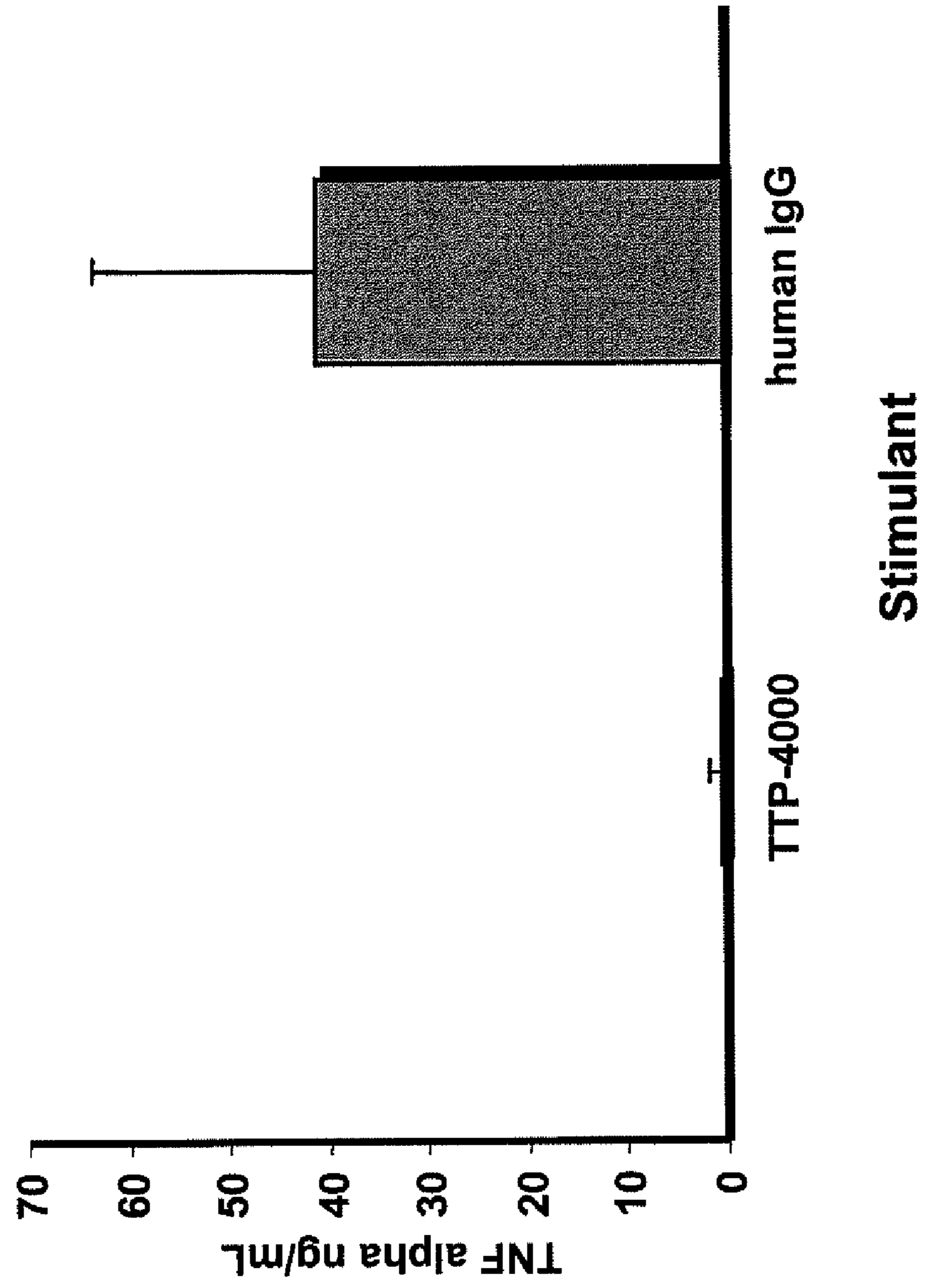


FIG. 14A

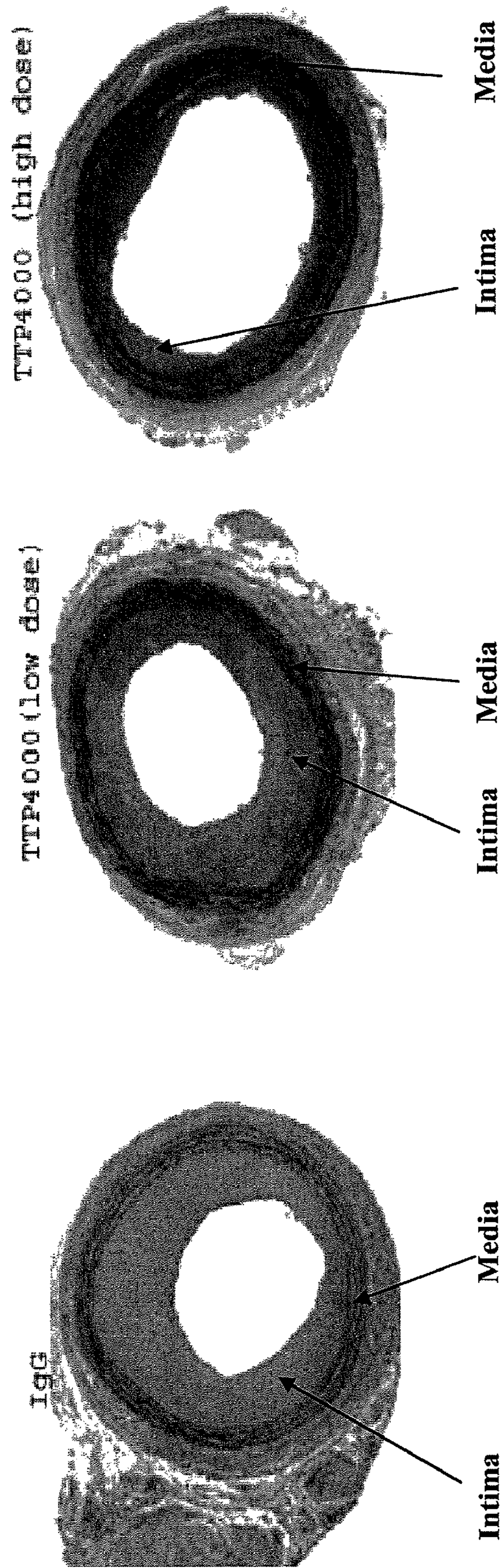


FIG. 14B

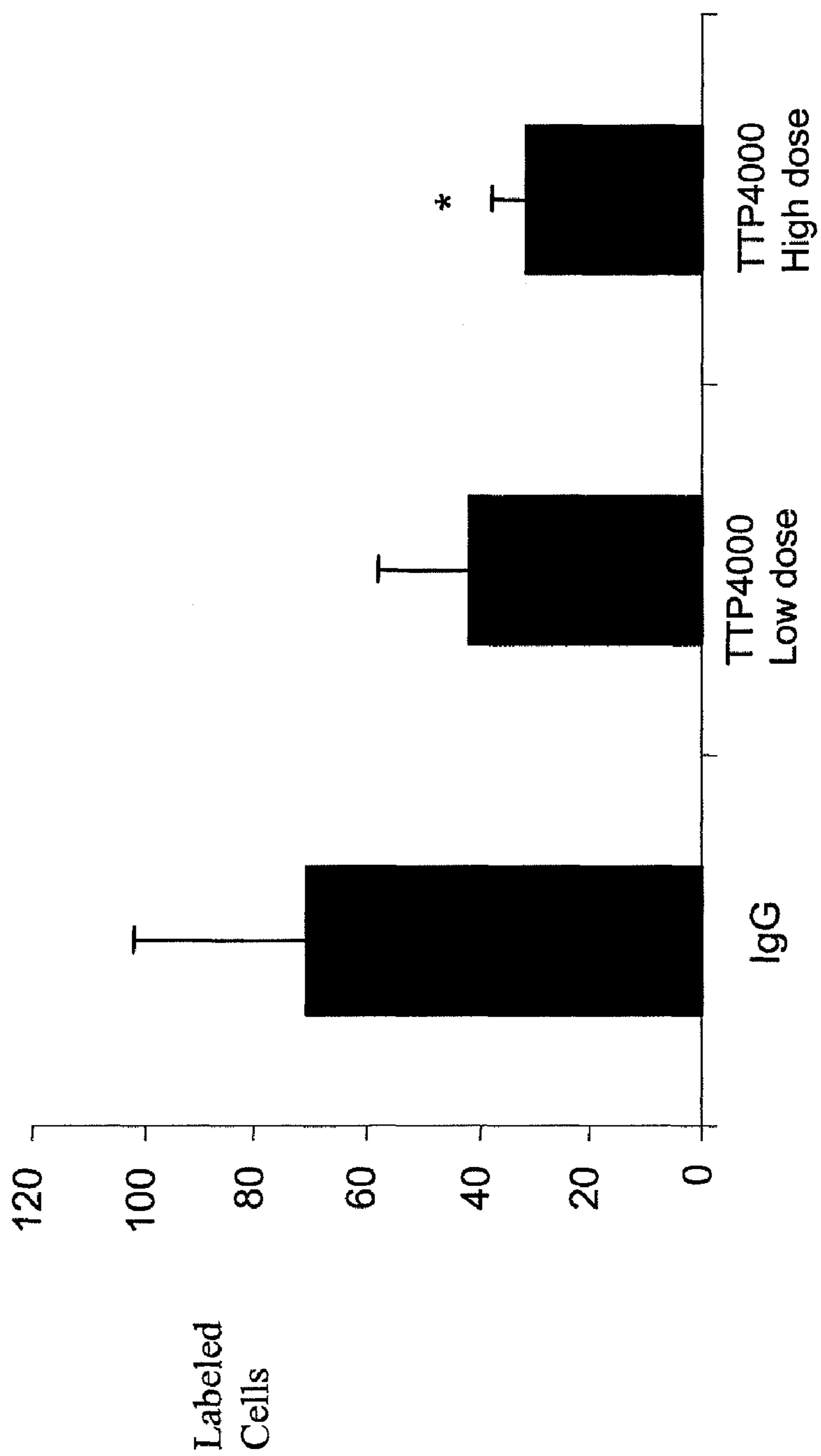


FIG. 15A

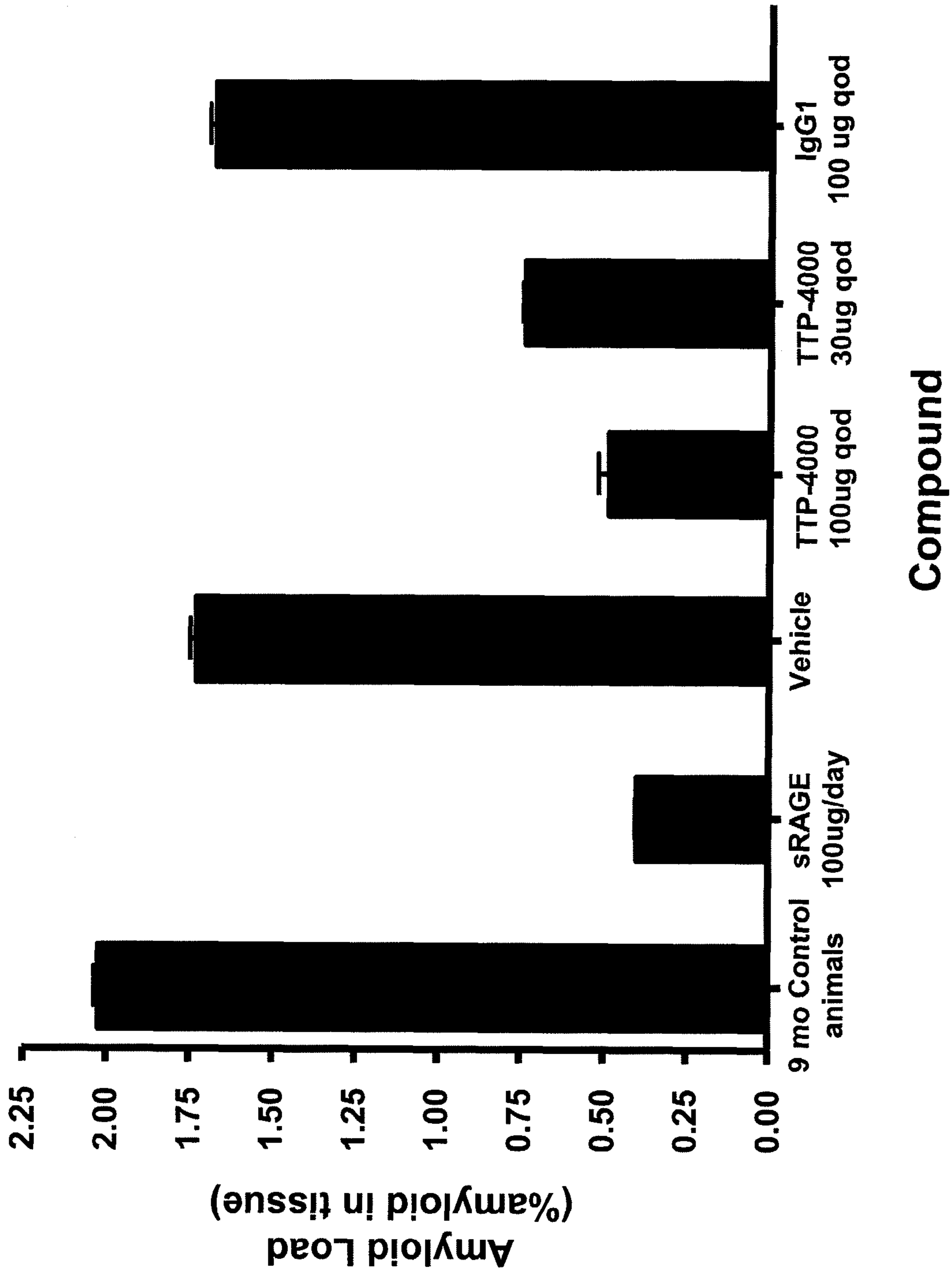


FIG. 15B

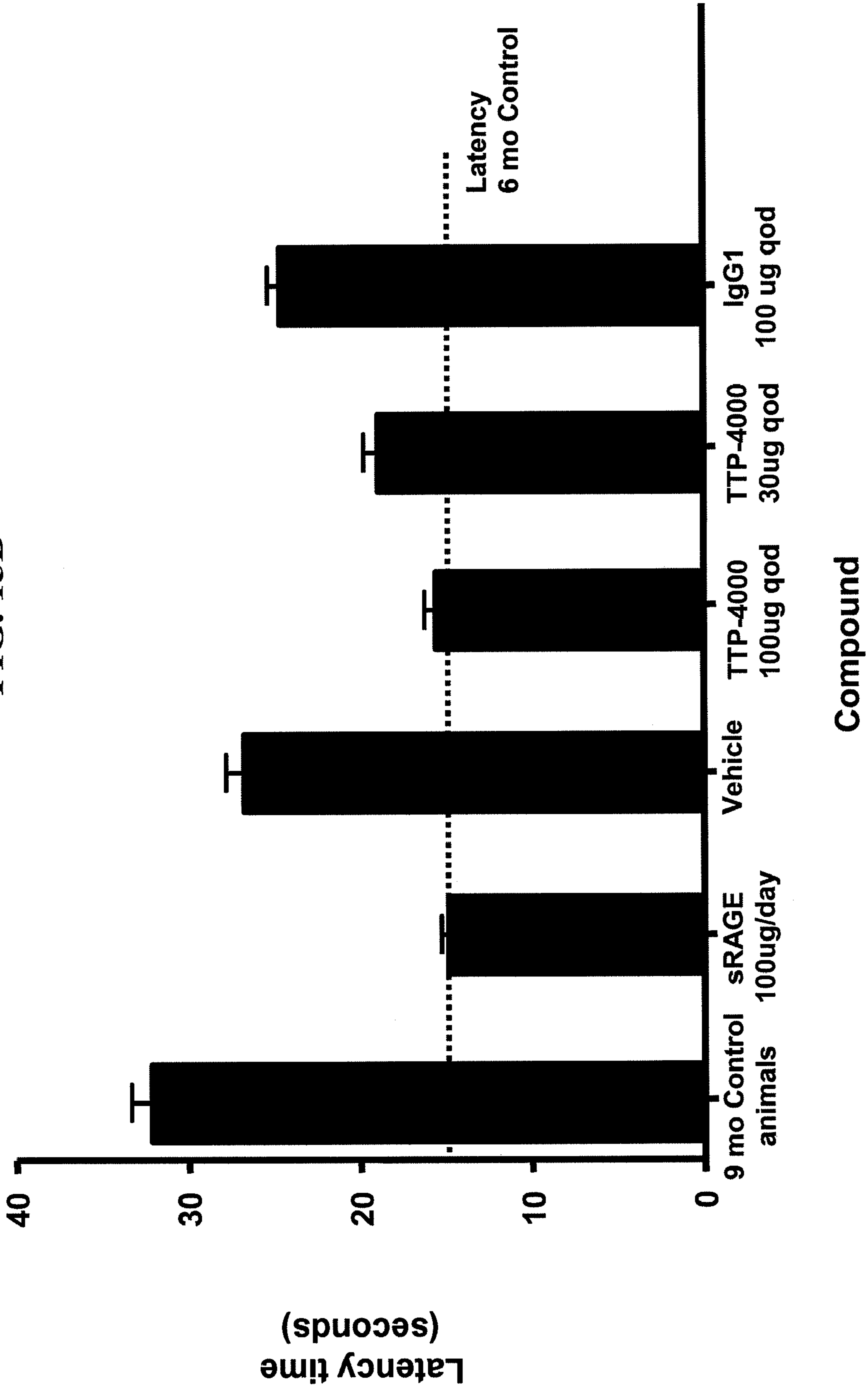
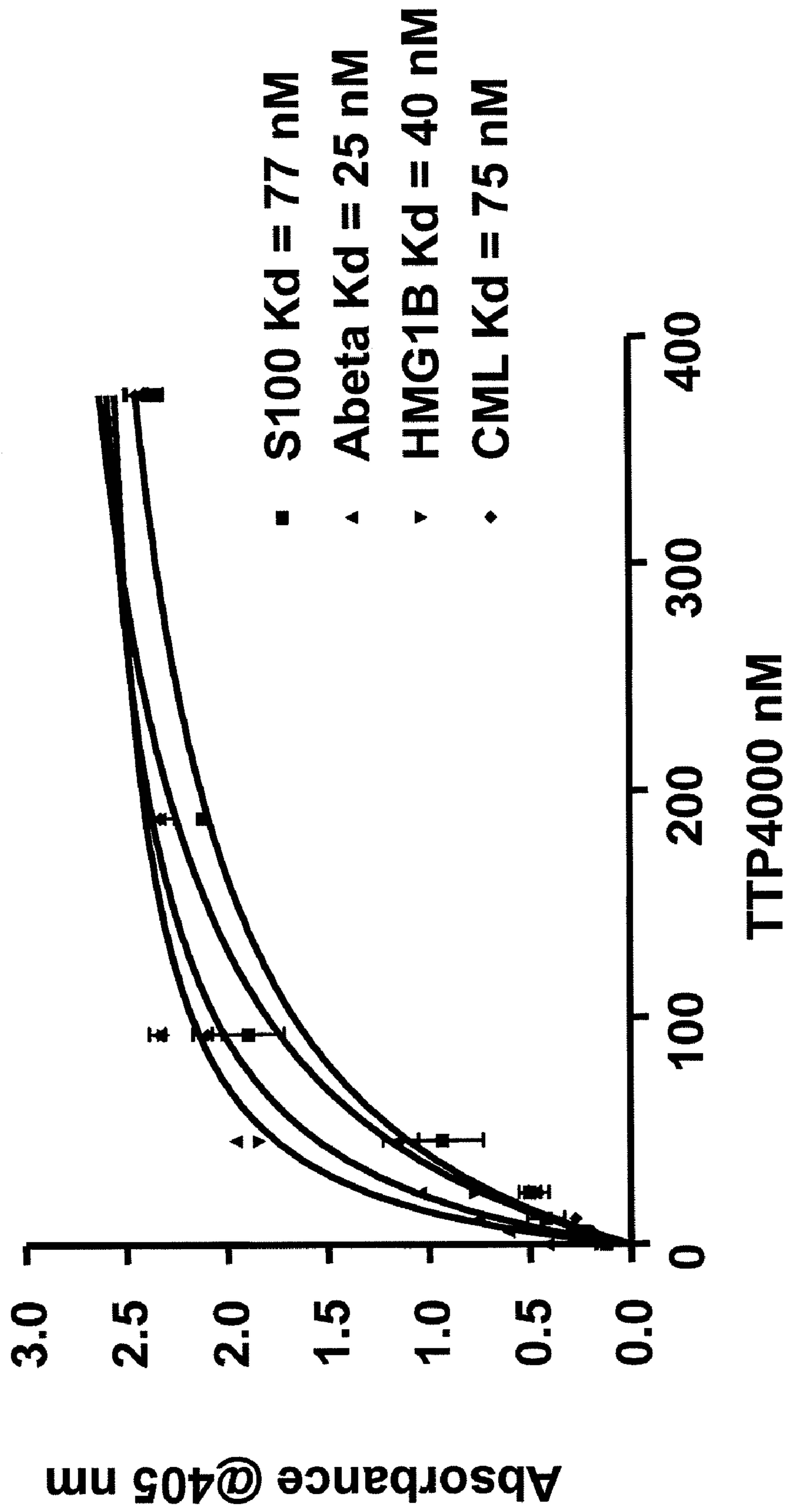


FIG. 16



Effects of TTP4000 on Rejection of Islet Allografts (BALB/c into B6)

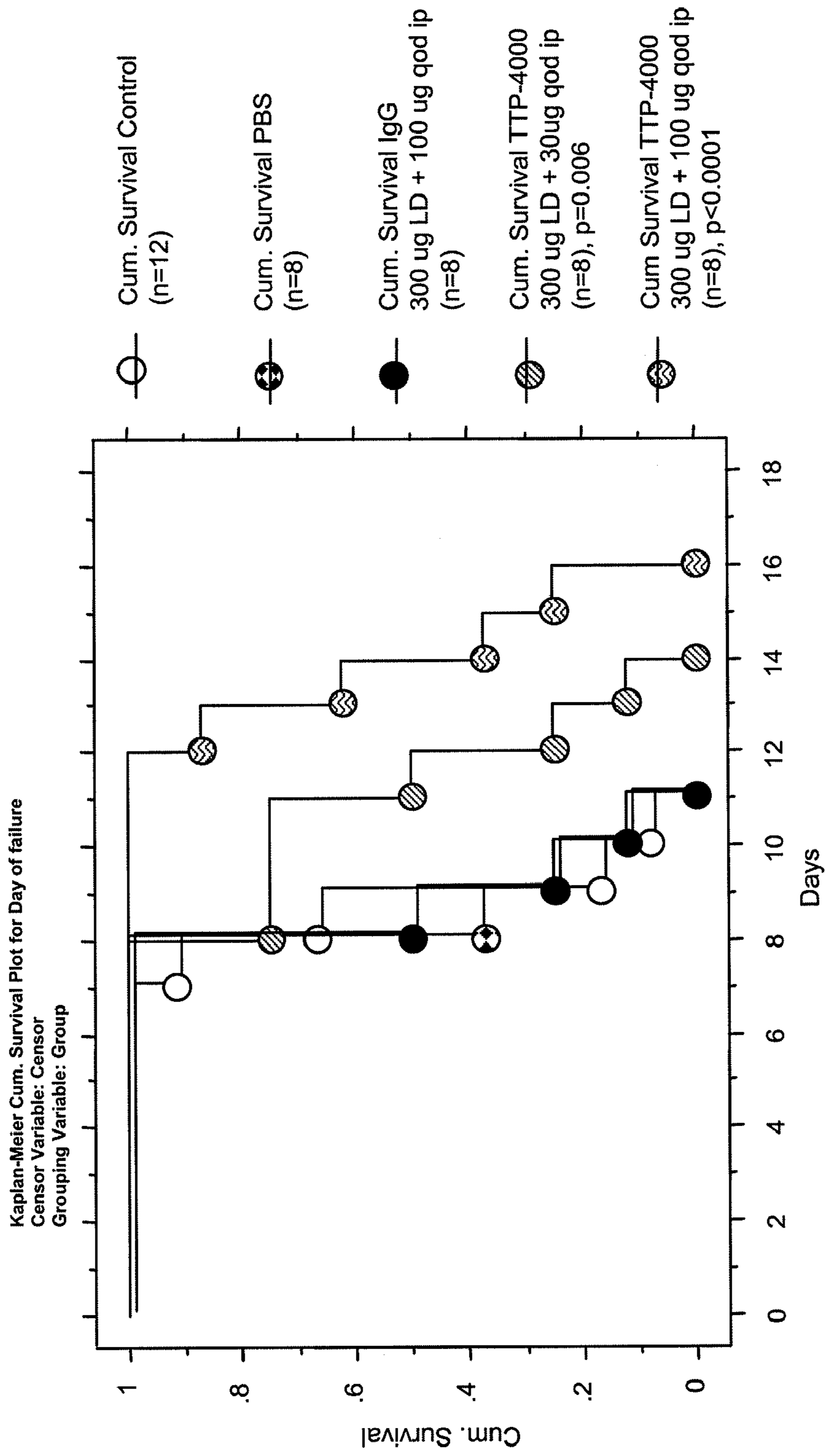


FIG. 17

Effects of TTP-4000 Treatment on Recurrent Diabetes in Transplanted Islets in Diabetic NOD Mice

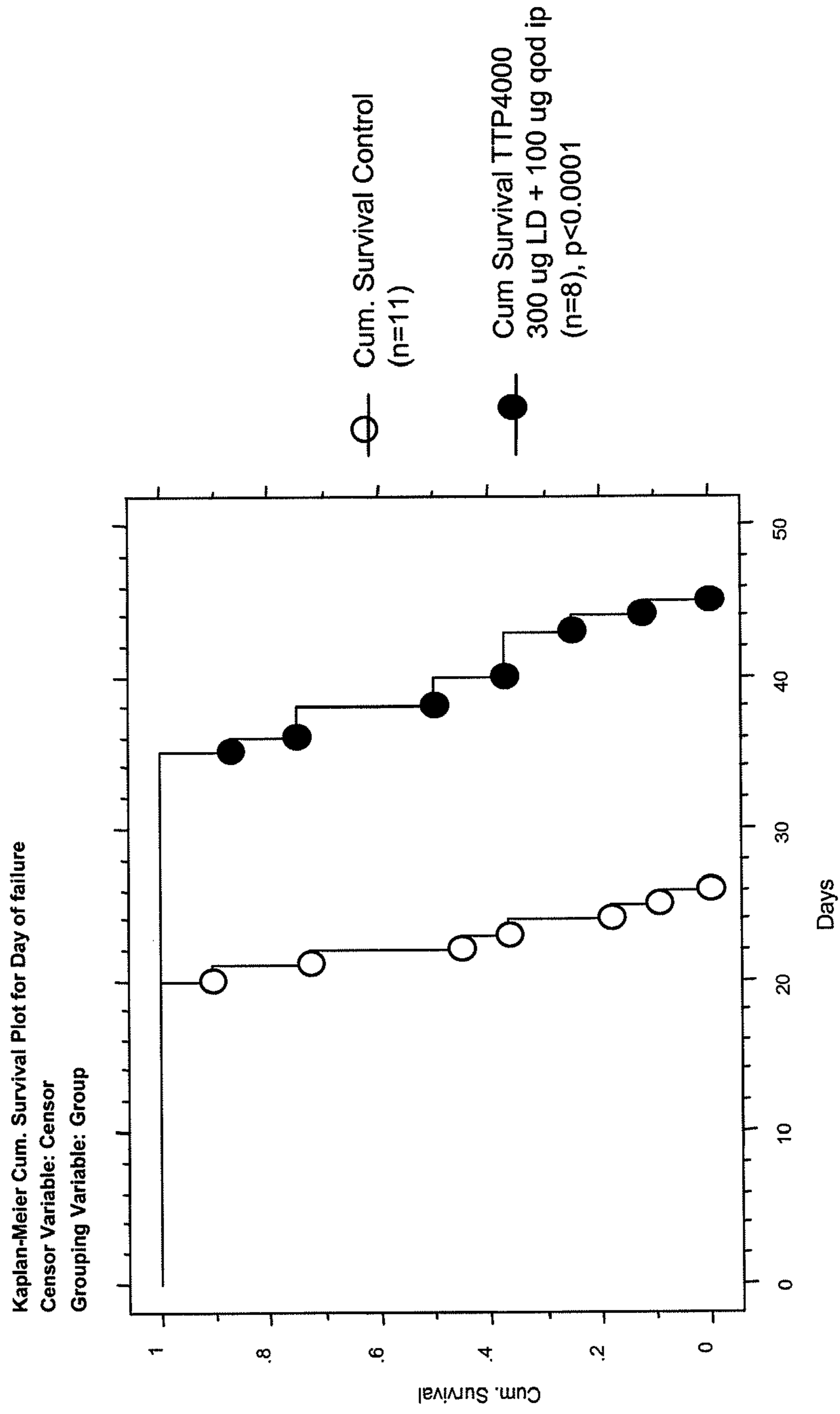


FIG. 18

NUCLEIC ACID MOLECULES ENCODING RAGE FUSION PROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a divisional application of U.S. patent application Ser. No. 11/789,637, filed Apr. 25, 2007, entitled "Rage Fusion Proteins, Formulations and Methods of Use Thereof", which claimed priority under 35 USC 119(e) from U.S. Provisional Patent Application No. 60/798,455, filed May 5, 2006. The disclosures of U.S. Provisional Patent Application No. 60/798,455 and U.S. patent application Ser. No. 11/789,637 are hereby incorporated by reference in their entireties herein.

FIELD OF THE INVENTION

The present invention relates to regulation of the Receptor for Advanced Glycated Endproducts (RAGE). More particularly, the present invention describes fusion proteins comprising a RAGE polypeptide, methods of making such fusion proteins and formulations of such RAGE fusion proteins, and the use of such RAGE fusion proteins for treatment of RAGE-based disorders.

BACKGROUND

Incubation of proteins or lipids with aldose sugars results in nonenzymatic glycation and oxidation of amino groups on proteins to form Amadori adducts. Over time, the adducts undergo additional rearrangements, dehydrations, and cross-linking with other proteins to form complexes known as Advanced Glycation End Products (AGEs). Factors which promote formation of AGEs include delayed protein turnover (e.g. as in amyloidoses), accumulation of macromolecules having high lysine content, and high blood glucose levels (e.g. as in diabetes) (Hori et al., *J. Biol. Chem.* 270: 25752-761, (1995)). AGEs have been implicated in a variety of disorders including complications associated with diabetes and normal aging.

AGEs display specific and saturable binding to cell surface receptors on monocytes, macrophages, endothelial cells of the microvasculature, smooth muscle cells, mesengial cells, and neurons. The Receptor for Advanced Glycated Endproducts (RAGE) is a member of the immunoglobulin supergene family of molecules. The extracellular (N-terminal) domain of RAGE includes three immunoglobulin-type regions: one V (variable) type domain followed by two C-type (constant) domains (Neeper et al., *J. Biol. Chem.*, 267:14998-15004 (1992); Schmidt et al., *Circ. (Suppl.)* 96#194 (1997)).

A single transmembrane spanning domain and a short, highly charged cytosolic tail follow the extracellular domain. The N-terminal, extracellular domain can be isolated by proteolysis of RAGE or by molecular biological approaches to generate soluble RAGE (sRAGE) comprised of the V and C domains.

RAGE is expressed on multiple cell types including leukocytes, neurons, microglial cells and vascular endothelium (e.g., Hon et al., *J. Biol. Chem.*, 270:25752-761 (1995)). Increased levels of RAGE are also found in aging tissues (Schleicher et al., *J. Clin. Invest.*, 99 (3): 457-468 (1997)), and the diabetic retina, vasculature and kidney (Schmidt et al., *Nature Med.*, 1:1002-1004 (1995)).

In addition to AGEs, other compounds can bind to and modulate RAGE. RAGE binds to multiple functionally and structurally diverse ligands including amyloid beta (A β),

serum amyloid A (SAA), Advanced Glycation End products (AGEs), S100 (a proinflammatory member of the Calgranulin family), carboxymethyl lysine (CML), amphoterin and CD11b/CD18 (Bucciarelli et al., *Cell Mol. Life Sci.*, 59:1117-128 (2002); Chavakis et al., *Microbes Infect.*, 6:1219-1225 (2004); Kokkola et al., *Scand. J. Immunol.*, 61:1-9 (2005); Schmidt et al., *J. Clin. Invest.*, 108:949-955 (2001); Rocken et al., *Am. J. Pathol.*, 162:1213-1220 (2003)).

Binding of ligands such as AGEs, S100/calgranulin, β -amyloid, CML (N^ε-Carboxymethyl lysine), and amphoterin to RAGE has been shown to modify expression of a variety of genes. These interactions may then initiate signal transduction mechanisms including p38 activation, p21ras, MAP kinases, Erk1-2 phosphorylation, and the activation of the transcriptional mediator of inflammatory signaling, NF- κ B (Yeh et al., *Diabetes*, 50:1495-1504 (2001)). For example, in many cell types, interaction between RAGE and its ligands can generate oxidative stress, which thereby results in activation of the free radical sensitive transcription factor NF- κ B, and the activation of NF- κ B regulated genes, such as the cytokines IL-1 β and TNF- α . Furthermore, RAGE expression is upregulated via NF- κ B and shows increased expression at sites of inflammation or oxidative stress (Tanaka et al., *J. Biol. Chem.*, 275:25781-25790 (2000)). Thus, an ascending and often detrimental spiral may be fueled by a positive feedback loop initiated by ligand binding.

Activation of RAGE in different tissues and organs can lead to a number of pathophysiological consequences. RAGE has been implicated in a variety of conditions including: acute and chronic inflammation (Hofmann et al., *Cell* 97:889-901 (1999)), the development of diabetic late complications such as increased vascular permeability (Wautier et al., *J. Clin. Invest.*, 97:238-243 (1995)), nephropathy (Teillet et al., *J. Am. Soc. Nephrol.*, 11:1488-1497 (2000)), arteriosclerosis (Vlasara et al., *The Finnish Medical Society DUODECIM, Ann. Med.*, 28:419-426 (1996)), and retinopathy (Hammes et al., *Diabetologia*, 42:603-607 (1999)). RAGE has also been implicated in Alzheimer's disease (Yan et al., *Nature*, 382: 685-691 (1996)), and in tumor invasion and metastasis (Taguchi et al., *Nature*, 405:354-357 (2000)).

Despite the broad expression of RAGE and its apparent pleiotropic role in multiple diverse disease models, RAGE does not appear to be essential to normal development. For example, RAGE knockout mice are without an overt abnormal phenotype, suggesting that while RAGE can play a role in disease pathology when stimulated chronically, inhibition of RAGE does not appear to contribute to any unwanted acute phenotype (Liliensiek et al., *J. Clin. Invest.*, 113:1641-50 (2004)).

Antagonizing binding of physiological ligands to RAGE may down-regulate the pathophysiological changes brought about by excessive concentrations of AGEs and other RAGE ligands. By reducing binding of endogenous ligands to RAGE, symptoms associated with RAGE-mediated disorders may be reduced. Soluble RAGE (sRAGE) is able to effectively antagonize the binding of RAGE ligands to RAGE. However, sRAGE can have a half-life when administered in vivo that may be too short to be therapeutically useful for one or more disorders. Thus, there is a need to develop compounds that antagonize the binding of AGEs and other physiological ligands to the RAGE receptor where the compound has a desirable pharmacokinetic profile.

SUMMARY

Embodiments of the present invention comprise RAGE fusion proteins and methods of using such proteins. The

present invention may be embodied in a variety of ways. Embodiments of the present invention may comprise a RAGE fusion protein comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the RAGE fusion protein comprises a RAGE ligand binding site. The RAGE fusion protein may further comprise a RAGE polypeptide directly linked to a polypeptide comprising the C_H2 domain of an immunoglobulin, or a portion of the C_H2 domain. In certain embodiments, the RAGE fusion protein comprises an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine.

The present invention also comprises a method to make a RAGE fusion protein. In one embodiment the method comprises linking a RAGE polypeptide to a second, non-RAGE polypeptide. In one embodiment, the RAGE polypeptide comprises a RAGE ligand binding site. The method may comprise linking a RAGE polypeptide directly to a polypeptide comprising the C_H2 domain of an immunoglobulin or a portion of the C_H2 domain. In certain embodiments, the RAGE fusion protein comprises an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine.

In other embodiments, the present invention may comprise methods and compositions for treating a RAGE-mediated disorder in a subject. The method may comprise administering a RAGE fusion protein of the present invention to the subject. The composition may comprise a RAGE fusion protein of the present invention in a pharmaceutically acceptable carrier.

In other embodiments, the present invention also provides formulations comprising a lyophilized mixture of a lyoprotectant, a RAGE fusion protein, and buffer. For example, in certain embodiments, the present invention may comprise a stable reconstituted formulation comprising a RAGE fusion protein in an amount of at least 50 mg/mL, and a diluent, where the reconstituted formulation has been prepared from a lyophilized mixture of the RAGE fusion protein and a lyoprotectant.

Embodiments of the present invention may also comprise articles of manufacture. In certain embodiments, the articles of manufacture may comprise a container which holds a formulation comprising a lyophilized RAGE fusion protein. The article of manufacture may also comprise instructions for reconstituting the lyophilized formulation with a diluent.

In other embodiments, the present invention may also comprise methods for preparing a stable reconstituted formulation of a RAGE fusion protein. In certain embodiments, the method may comprise reconstituting a lyophilized mixture of a RAGE fusion protein and a lyoprotectant in a diluent such that the RAGE fusion protein concentration in the reconstituted formulation is at least 50 mg/mL. For example, in one embodiment, the method may comprise the steps of lyophilizing a mixture comprising a RAGE fusion protein and a lyoprotectant amount of a lyoprotectant, and reconstituting the lyophilized mixture in a diluent.

There are various advantages that may be associated with particular embodiments of the present invention. In one embodiment, the RAGE fusion proteins of the present inven-

tion may be metabolically stable when administered to a subject. Also, the RAGE fusion proteins of the present invention may exhibit high-affinity binding for RAGE ligands. In certain embodiments, the RAGE fusion proteins of the present invention bind to RAGE ligands with affinities in the high nanomolar to low micromolar range. By binding with high affinity to physiological RAGE ligands, the RAGE fusion proteins of the present invention may be used to inhibit binding of endogenous ligands to RAGE, thereby providing a means to ameliorate RAGE-mediated diseases.

Also, the RAGE fusion proteins of the present invention may be provided in protein or nucleic acid form. In one example embodiment, the RAGE fusion protein may be administered systemically and remain in the vasculature to potentially treat vascular diseases mediated in part by RAGE. In another example embodiment, the RAGE fusion protein may be administered locally to treat diseases where RAGE ligands contribute to the pathology of the disease. Alternatively, a nucleic acid construct encoding the RAGE fusion protein may be delivered to a site by the use of an appropriate carrier such as a virus, or as a naked DNA, where transient local expression may locally inhibit the interaction between RAGE ligands and receptors. Thus, administration may be transient (e.g., as where the RAGE fusion protein is administered) or more permanent in nature (e.g., as where the RAGE fusion protein is administered as a recombinant DNA).

There are additional features of the invention which will be described hereinafter. It is to be understood that the invention is not limited in its application to the details set forth in the following claims, description and figures. The invention is capable of other embodiments and of being practiced or carried out in various ways.

BRIEF DESCRIPTION OF THE FIGURES

Various features, aspects and advantages of the present invention will become more apparent with reference to the following figures.

FIG. 1 shows various RAGE sequences and immunoglobulin sequences in accordance with alternate embodiments of the present invention: Panel A, SEQ ID NO: 1, the amino acid sequence for human RAGE; and SEQ ID NO: 2, the amino acid sequence for human RAGE without the signal sequence of amino acids 1-22; Panel B, SEQ ID NO: 3, the amino acid sequence for human RAGE without the signal sequence of amino acids 1-23; Panel C, SEQ ID NO: 4, the amino acid sequence of human sRAGE; SEQ ID NO: 5, the amino acid sequence of human sRAGE without the signal sequence of amino acids 1-22, and SEQ ID NO: 6, the amino acid sequence of human sRAGE without the signal sequence of amino acids 1-23; Panel D, SEQ ID NO: 7, an amino acid sequence comprising the V-domain of human RAGE; SEQ ID NO: 8, an alternate amino acid sequence comprising the V-domain of human RAGE; SEQ ID NO: 9, an N-terminal fragment of the V-domain of human RAGE; SEQ ID NO: 10, an alternate N-terminal fragment of the V-domain of human RAGE; SEQ ID NO: 11, the amino acid sequence for amino acids 124-221 of human RAGE; SEQ ID NO: 12, the amino acid sequence for amino acids 227-317 of human RAGE; SEQ ID NO: 13, the amino acid sequence for amino acids 23-123 of human RAGE; Panel E, SEQ ID NO: 14, the amino acid sequence for amino acids 24-123 of human RAGE; SEQ ID NO: 15, the amino acid sequence for amino acids 23-136 of human RAGE; SEQ ID NO: 16, the amino acid sequence for amino acids 24-136 of human RAGE; SEQ ID NO: 17, the amino acid sequence for amino acids 23-226 of human

5

RAGE; SEQ ID NO: 18, the amino acid sequence for amino acids 24-226 of human RAGE; Panel F, SEQ ID NO: 19, the amino acid sequence for amino acids 23-251 of human RAGE; SEQ ID NO: 20, the amino acid sequence for amino acids 24-251 of human RAGE; SEQ ID NO: 21, a RAGE interdomain linker; SEQ ID NO: 22, a second RAGE interdomain linker; SEQ ID NO: 23, a third RAGE interdomain linker; SEQ ID NO: 24, a fourth RAGE interdomain linker; Panel G, SEQ ID NO: 25, DNA encoding human RAGE amino acids 1-118; SEQ ID NO: 26, DNA encoding human RAGE amino acids 1-123; and SEQ ID NO: 27, DNA encoding human RAGE amino acids 1-136; Panel H, SEQ ID NO: 28, DNA encoding human RAGE amino acids 1-230; and SEQ ID NO: 29, DNA encoding human RAGE amino acids 1-251; Panel I, SEQ ID NO: 38, a partial amino acid sequence for the C_{H2} and C_{H3} domains of human IgG; SEQ ID NO: 39, DNA encoding a portion of the human C_{H2} and C_{H3} domains of human IgG; SEQ ID NO: 40, an amino acid sequence for the C_{H2} and C_{H3} domains of human IgG; Panel J, SEQ ID NO: 41, a DNA encoding the human C_{H2} and C_{H3} domains of human IgG; SEQ ID NO: 42, an amino acid sequence for the C_{H2} domain of human IgG; SEQ ID NO: 43, an amino acid sequence for the C_{H3} domain of human IgG; and SEQ ID NO: 44, a fifth RAGE interdomain linker; Panel K, SEQ ID NO: 45, the amino acid sequence of human sRAGE without the signal sequence of amino acids 1-23 where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid, SEQ ID NO: 46, an alternate amino acid sequence comprising the V-domain of human sRAGE where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid, SEQ ID NO: 47, an alternate N-terminal fragment of the V-domain of human RAGE where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid, SEQ ID NO: 48, the amino acid sequence for amino acids 24-123 of human RAGE where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid; Panel L, SEQ ID NO: 49, the amino acid sequence for amino acids 24-136 of human RAGE where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid, SEQ ID NO: 50, the amino acid sequence for amino acids 24-226 of human RAGE where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid, SEQ ID NO: 51, the amino acid sequence for amino acids 24-251 of human RAGE where the glutamine residue at the N-terminus has cyclized to form pyroglutamic acid; Panel M, SEQ ID NO: 52, an alternate DNA sequence encoding a portion of the human C_{H2} and C_{H3} domains of human IgG in SEQ ID NO: 38, and SEQ ID NO: 53, an alternate DNA sequence encoding the human C_{H2} and C_{H3} domains of human IgG in SEQ ID NO: 40.

FIG. 2 shows alternate DNA sequences SEQ ID NO: 30 (Panel A) and SEQ ID NO: 54 (Panel B) that encode a first RAGE fusion protein (TTP-4000) coding region in accordance with an embodiment of the present invention. Coding sequence 1-753 highlighted in bold encodes RAGE N-terminal protein sequence whereas sequence 754-1386 encodes human IgG Fc ($\gamma 1$) protein sequence without the hinge region.

FIG. 3 shows alternate DNA sequences SEQ ID NO: 31 (Panel A) and SEQ ID NO: 55 (Panel B) that encode a second RAGE fusion protein (TTP-3000) coding region in accordance with an embodiment of the present invention. Coding sequence 1-408 highlighted in bold encodes RAGE N-terminal protein sequence, whereas sequence 409-1041 codes human IgG Fc ($\gamma 1$) protein sequence without the hinge region.

FIG. 4 shows the amino acid sequences, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, and SEQ ID NO: 56 that each encode a four domain RAGE fusion protein in accordance

6

with alternate embodiments of the present invention. RAGE sequence is highlighted with bold font.

FIG. 5 shows the amino acid sequences, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 57 that each encode a three domain RAGE fusion protein in accordance with alternate embodiments of the present invention. RAGE sequence is highlighted with bold font.

FIG. 6, Panel A, shows a comparison of the protein domains in human RAGE and human Ig gamma-1 Fc protein, and cleavage points used to make TTP-3000 (at position 136) and TTP-4000 (at position 251) in accordance with alternate embodiments of the present invention; and Panel B shows the domain structure for TTP-3000 and TTP-4000 in accordance with alternate embodiments of the present invention.

FIG. 7 shows results of an in vitro binding assay for sRAGE, and a first RAGE fusion protein TTP-4000 (TT4) and a second RAGE fusion protein TTP-3000 (TT3), to the RAGE ligands amyloid-beta (A-beta), S100b (S100), and amphotericin (Ampho), in accordance with an embodiment of the present invention.

FIG. 8 shows results of an in vitro binding assay for a first RAGE fusion protein TTP-4000 (TT4) ("Protein") to amyloid-beta as compared to a negative control only including the immunodetection reagents ("Complex Alone"), and antagonism of such binding by a RAGE antagonist ("RAGE Ligand") in accordance with an embodiment of the present invention.

FIG. 9 shows results of an in vitro binding assay for a second RAGE fusion protein TTP-3000 (TT3) ("Protein") to amyloid-beta as compared to a negative control only including the immunodetection reagents ("Complex Alone"), and antagonism of such binding by a RAGE antagonist ("RAGE Ligand") in accordance with an embodiment of the present invention.

FIG. 10 shows results of a cell-based assay measuring the inhibition of S100b-RAGE induced production of TNF- α by RAGE fusion proteins TTP-3000 (TT3) and TTP-4000 (TT4), and sRAGE in accordance with an embodiment of the present invention.

FIG. 11 shows results of a cell-based assay measuring the inhibition of HMGB1-RAGE induced production of TNF- α by RAGE fusion protein TTP-4000 and an anti-RAGE antibody in accordance with an embodiment of the present invention.

FIG. 12 shows a pharmacokinetic profile for RAGE fusion protein TTP-4000 in accordance with an embodiment of the present invention wherein each curve represents a different animal under the same experimental conditions.

FIG. 13 shows relative levels of TNF- α release from THP-1 cells due to stimulation by RAGE fusion protein TTP-4000 and human IgG stimulation as a measure of an inflammatory response in accordance with an embodiment of the present invention.

FIG. 14 shows the use of RAGE fusion protein TTP-4000 to reduce restenosis in diabetic animals in accordance with alternate embodiments of the present invention, wherein panel A shows that TTP-4000 RAGE-fusion protein reduced the intima/media ratio as compared to a negative control (IgG), and panel B shows that TTP-4000 RAGE-fusion protein reduced vascular smooth muscle cell proliferation in a dose-responsive manner.

FIG. 15 shows use of RAGE fusion protein TTP-4000 to reduce amyloid formation and cognitive dysfunction in animals with Alzheimer's Disease (AD) in accordance with alternate embodiments of the present invention wherein panel A shows TTP-4000 RAGE-fusion protein reduced amyloid

load in the brain, and panel B shows TTP-4000 RAGE-fusion protein improved cognitive function.

FIG. 16 shows saturation-binding curves with TTP-4000 to various immobilized known RAGE ligands in accordance with an embodiment of the present invention.

FIG. 17 shows the use of RAGE fusion protein TTP-4000 to reduce the rejection of allogeneic pancreatic islet cell transplants in accordance with alternate embodiments of the present invention where open (unfilled) circles designate untreated control animals; circles with diagonal hatching designate animals treated with TTP-4000 at a first dosage; circles with wavy hatching designate animals treated with TTP-4000 at a second dosage; diamond-filled circles designate animals treated with control PBS; and solid circles designate animals treated with control IgG.

FIG. 18 shows the use of RAGE fusion proteins TTP-4000 to reduce the rejection of syngeneic pancreatic islet cell transplants in accordance with alternate embodiments of the present invention where open (unfilled) circles designate untreated control animals; and solid circles designate animals treated with TTP-4000.

DETAILED DESCRIPTION

Unless indicated to the contrary, the numerical parameters set forth in the following specification are approximations that can vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Moreover, all ranges disclosed herein are to be understood to encompass any and all subranges subsumed therein. For example, a stated range of "1 to 10" should be considered to include any and all subranges between (and inclusive of) the minimum value of 1 and the maximum value of 10; that is, all subranges beginning with a minimum value of 1 or more, e.g. 1 to 6.1, and ending with a maximum value of 10 or less, e.g., 5.5 to 10. Additionally, any reference referred to as being "incorporated herein" is to be understood as being incorporated in its entirety.

It is further noted that, as used in this specification, the singular forms "a," "an," and "the" include plural referents unless expressly and unequivocally limited to one referent. The term "or" is used interchangeably with the term "and/or" unless the context clearly indicates otherwise.

Also, the terms "portion" and "fragment" are used interchangeably to refer to parts of a polypeptide, nucleic acid, or other molecular construct.

"Polypeptide" and "protein" are used interchangeably herein to describe protein molecules that may comprise either partial or full-length proteins.

As is known in the art, "proteins," "peptides," "polypeptides" and "oligopeptides" are chains of amino acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the alpha carbon of one amino acid and the amino group of the alpha carbon of another amino acid. Typically, the amino acids making up a protein are numbered

in order, starting at the amino terminal residue and increasing in the direction toward the carboxy terminal residue of the protein.

As used herein, the term "upstream" refers to a residue that is N-terminal to a second residue where the molecule is a protein, or 5' to a second residue where the molecule is a nucleic acid. Also as used herein, the term "downstream" refers to a residue that is C-terminal to a second residue where the molecule is a protein, or 3' to a second residue where the molecule is a nucleic acid.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Practitioners are particularly directed to Current Protocols in Molecular Biology (see e.g. Ausubel, F. M. et al., *Short Protocols in Molecular Biology*, 4th Ed., Chapter 2, John Wiley & Sons, N.Y.) for definitions and terms of the art. Abbreviations for amino acid residues are the standard 3-letter and/or 1-letter codes used in the art to refer to one of the 20 common L-amino acids.

A "nucleic acid" is a polynucleotide such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The term is used to include single-stranded nucleic acids, double-stranded nucleic acids, and RNA and DNA made from nucleotide or nucleoside analogues.

The term "vector" refers to a nucleic acid molecule that may be used to transport a second nucleic acid molecule into a cell. In one embodiment, the vector allows for replication of DNA sequences inserted into the vector. The vector may comprise a promoter to enhance expression of the nucleic acid molecule in at least some host cells. Vectors may replicate autonomously (extrachromosomal) or may be integrated into a host cell chromosome. In one embodiment, the vector may comprise an expression vector capable of producing a protein derived from at least part of a nucleic acid sequence inserted into the vector.

As is known in the art, conditions for hybridizing nucleic acid sequences to each other can be described as ranging from low to high stringency. Generally, highly stringent hybridization conditions refer to washing hybrids in low salt buffer at high temperatures. Hybridization may be to filter bound DNA using hybridization solutions standard in the art such as 0.5M NaHPO₄, 7% sodium dodecyl sulfate (SDS), at 65° C., and washing in 0.25 M NaHPO₄, 3.5% SDS followed by washing 0.1×SSC/0.1% SDS at a temperature ranging from room temperature to 68° C. depending on the length of the probe. For example, a high stringency wash comprises washing in 6×SSC/0.05% sodium pyrophosphate at 37° C. for a 14 base oligonucleotide probe, or at 48° C. for a 17 base oligonucleotide probe, or at 55° C. for a 20 base oligonucleotide probe, or at 60° C. for a 25 base oligonucleotide probe, or at 65° C. for a nucleotide probe about 250 nucleotides in length. Nucleic acid probes may be labeled with radionucleotides by end-labeling with, for example, [γ -³²P]ATP, or incorporation of radiolabeled nucleotides such as [α -³²P]dCTP by random primer labeling. Alternatively, probes may be labeled by incorporation of biotinylated or fluorescein labeled nucleotides, and the probe detected using Streptavidin or anti-fluorescein antibodies.

As used herein, "small organic molecules" are molecules of molecular weight less than 2,000 Daltons that contain at least one carbon atom.

The term "fusion protein" refers to a protein or polypeptide that has an amino acid sequence derived from two or more proteins. The fusion protein may also include linking regions of amino acids between amino acid portions derived from separate proteins.

As used herein, a “non-RAGE polypeptide” is any polypeptide that is not derived from RAGE or a fragment thereof. Such non-RAGE polypeptides include immunoglobulin peptides, dimerizing polypeptides, stabilizing polypeptides, amphiphilic peptides, or polypeptides comprising amino acid sequences that provide “tags” for targeting or purification of the protein.

As used herein, “immunoglobulin peptides” may comprise an immunoglobulin heavy chain or a portion thereof. In one embodiment, the portion of the heavy chain may be the Fc fragment or a portion thereof. As used herein, the Fc fragment comprises the heavy chain hinge polypeptide, and the C_{H2} and C_{H3} domains of the heavy chain of an immunoglobulin, in either monomeric or dimeric form. Or, the C_{H1} and Fc fragment may be used as the immunoglobulin polypeptide. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ϵ), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 ($\gamma 1$), IgG2 ($\gamma 2$), IgG3 ($\gamma 3$), IgG4 ($\gamma 4$), IgA1 ($\alpha 1$), IgA2 ($\alpha 2$), or mutations of these isotypes or subtypes that alter the biological activity. An example of biological activity that may be altered includes reduction of an isotype’s ability to bind to some Fc receptors as for example, by modification of the hinge region.

The terms “identity” or “percent identical” refers to sequence identity between two amino acid sequences or between two nucleic acid sequences. Percent identity can be determined by aligning two sequences and refers to the number of identical residues (i.e., amino acid or nucleotide) at positions shared by the compared sequences. Sequence alignment and comparison may be conducted using the algorithms standard in the art (e.g. Smith and Waterman, 1981, *Adv. Appl. Math.* 2:482; Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443; Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci., USA*, 85:2444) or by computerized versions of these algorithms (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive, Madison, Wis.) publicly available as BLAST and FASTA. Also, ENTREZ, available through the National Institutes of Health, Bethesda Md., may be used for sequence comparison. In one embodiment, the percent identity of two sequences may be determined using GCG with a gap weight of 1, such that each amino acid gap is weighted as if it were a single amino acid mismatch between the two sequences.

As used herein, the term “conserved residues” refers to amino acids that are the same among a plurality of proteins having the same structure and/or function. A region of conserved residues may be important for protein structure or function. Thus, contiguous conserved residues as identified in a three-dimensional protein may be important for protein structure or function. To find conserved residues, or conserved regions of 3-D structure, a comparison of sequences for the same or similar proteins from different species, or of individuals of the same species, may be made.

As used herein, the term “homologue” means a polypeptide having a degree of homology or identity with the wild-type amino acid sequence. Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percent homology between two or more sequences (e.g. Wilbur, W. J. and Lipman, D. J., 1983, *Proc. Natl. Acad. Sci. USA*, 80:726-730). For example, homologous sequences may be taken to include an amino acid sequences which in alternate embodiments are at least 70% identical, 75% identical, 85% identical,

90% identical, 95% identical, 96% identical, 97% identical, 98% identical, or 99% identical to each other.

As used herein, the term at least 90% identical thereto includes sequences that range from 90 to 99.99% identity to the indicated sequences and includes all ranges in between. Thus, the term at least 90% identical thereto includes sequences that are 91, 91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5 percent identical to the indicated sequence. Similarly the term “at least 70% identical includes sequences that range from 70 to 99.99% identical, with all ranges in between. The determination of percent identity is determined using the algorithms described here.

As used herein, a polypeptide or protein “domain” comprises a region along a polypeptide or protein that comprises an independent unit. Domains may be defined in terms of structure, sequence and/or biological activity. In one embodiment, a polypeptide domain may comprise a region of a protein that folds in a manner that is substantially independent from the rest of the protein. Domains may be identified using domain databases such as, but not limited to PFAM, PRODOM, PROSITE, BLOCKS, PRINTS, SBASE, ISREC PROFILES, SAMRT, and PROCLASS.

As used herein, “immunoglobulin domain” is a sequence of amino acids that is structurally homologous, or identical to, a domain of an immunoglobulin. The length of the sequence of amino acids of an immunoglobulin domain may be any length. In one embodiment, an immunoglobulin domain may be less than 250 amino acids. In an example embodiment, an immunoglobulin domain may be about 80-150 amino acids in length. For example, the variable region, and the C_{H1} , C_{H2} , and C_{H3} regions of an IgG are each immunoglobulin domains. In another example, the variable, the C_{H1} , C_{H2} , C_{H3} and C_{H4} regions of an IgM are each immunoglobulin domains.

As used herein, a “RAGE immunoglobulin domain” is a sequence of amino acids from RAGE protein that is structurally homologous, or identical to, a domain of an immunoglobulin. For example, a RAGE immunoglobulin domain may comprise the RAGE V-domain, the RAGE Ig-like C1-type 1 domain (“C1 domain”), or the RAGE Ig-like C2-type 2 domain (“C2 domain”).

As used herein, an “interdomain linker” comprises a polypeptide that joins two domains together. An Fc hinge region is an example of an interdomain linker in an IgG.

As used herein, “directly linked” identifies a covalent linkage between two different groups (e.g., nucleic acid sequences, polypeptides, polypeptide domains) that does not have any intervening atoms between the two groups that are being linked.

As used herein, “ligand binding domain” refers to a domain of a protein responsible for binding a ligand. The term ligand binding domain includes homologues of a ligand binding domain or portions thereof. In this regard, deliberate amino acid substitutions may be made in the ligand binding site on the basis of similarity in polarity, charge, solubility, hydrophobicity, or hydrophilicity of the residues, as long as the binding specificity of the ligand binding domain is retained.

As used herein, a “ligand binding site” comprises residues in a protein that directly interact with a ligand, or residues involved in positioning the ligand in close proximity to those residues that directly interact with the ligand. The interaction of residues in the ligand binding site may be defined by the spatial proximity of the residues to a ligand in the model or structure. The term ligand binding site includes homologues of a ligand binding site, or portions thereof. In this regard, deliberate amino acid substitutions may be made in the ligand

binding site on the basis of similarity in polarity, charge, solubility, hydrophobicity, or hydrophilicity of the residues, as long as the binding specificity of the ligand binding site is retained. A ligand binding site may exist in one or more ligand binding domains of a protein or polypeptide.

As used herein, the term “interact” refers to a condition of proximity between a ligand or compound, or portions or fragments thereof, and a portion of a second molecule of interest. The interaction may be non-covalent, for example, as a result of hydrogen-bonding, van der Waals interactions, or electrostatic or hydrophobic interactions, or it may be covalent.

As used herein, a “ligand” refers to a molecule or compound or entity that interacts with a ligand binding site, including substrates or analogues or parts thereof. As described herein, the term “ligand” may refer to compounds that bind to the protein of interest. A ligand may be an agonist, an antagonist, or a modulator. Or, a ligand may not have a biological effect. Or, a ligand may block the binding of other ligands thereby inhibiting a biological effect. Ligands may include, but are not limited to, small molecule inhibitors. These small molecules may include peptides, peptidomimetics, organic compounds and the like. Ligands may also include polypeptides and/or proteins.

As used herein, a “modulator compound” refers to a molecule which changes or alters the biological activity of a molecule of interest. A modulator compound may increase or decrease activity, or change the physical or chemical characteristics, or functional or immunological properties, of the molecule of interest. For RAGE, a modulator compound may increase or decrease activity, or change the characteristics, or functional or immunological properties of the RAGE, or a portion thereof. A modulator compound may include natural and/or chemically synthesized or artificial peptides, modified peptides (e.g., phosphopeptides), antibodies, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, glycolipids, heterocyclic compounds, nucleosides or nucleotides or parts thereof, and small organic or inorganic molecules. A modulator compound may be an endogenous physiological compound or it may be a natural or synthetic compound. Or, the modulator compound may be a small organic molecule. The term “modulator compound” also includes a chemically modified ligand or compound, and includes isomers and racemic forms.

An “agonist” comprises a compound that binds to a receptor to form a complex that elicits a pharmacological response specific to the receptor involved.

An “antagonist” comprises a compound that binds to an agonist or to a receptor to form a complex that does not give rise to a substantial pharmacological response and can inhibit the biological response induced by an agonist.

RAGE agonists may therefore bind to RAGE and stimulate RAGE-mediated cellular processes, and RAGE antagonists may inhibit RAGE-mediated processes from being stimulated by a RAGE agonist. For example, in one embodiment, the cellular process stimulated by RAGE agonists comprises activation of TNF- α gene transcription.

The term “peptide mimetics” refers to structures that serve as substitutes for peptides in interactions between molecules (Morgan et al., 1989, *Ann. Reports Med. Chem.*, 24:243-252). Peptide mimetics may include synthetic structures that may or may not contain amino acids and/or peptide bonds but that retain the structural and functional features of a peptide, or agonist, or antagonist. Peptide mimetics also include peptoids, oligopeptoids (Simon et al., 1972, *Proc. Natl. Acad. Sci., USA*, 89:9367); and peptide libraries containing peptides

of a designed length representing all possible sequences of amino acids corresponding to a peptide, or agonist or antagonist of the invention.

The term “treating” or “treat” refers to improving a symptom of a disease or disorder and may comprise curing the disorder, substantially preventing the onset of the disorder, or improving the subject’s condition. The term “treatment” as used herein, refers to the full spectrum of treatments for a given disorder from which the patient is suffering, including alleviation of one symptom or most of the symptoms resulting from that disorder, a cure for the particular disorder, or prevention of the onset of the disorder.

As used herein, the term “EC50” is defined as the concentration of an agent that results in 50% of a measured biological effect. For example, the EC50 of a therapeutic agent having a measurable biological effect may comprise the value at which the agent displays 50% of the biological effect.

As used herein, the term “IC50” is defined as the concentration of an agent that results in 50% inhibition of a measured effect. For example, the IC50 of an antagonist of RAGE binding may comprise the value at which the antagonist reduces ligand binding to the ligand binding site of RAGE by 50%.

As used herein, an “effective amount” means the amount of an agent that is effective for producing a desired effect in a subject. The term “therapeutically effective amount” denotes that amount of a drug or pharmaceutical agent that will elicit therapeutic response of an animal or human that is being sought. The actual dose which comprises the effective amount may depend upon the route of administration, the size and health of the subject, the disorder being treated, and the like.

The term “pharmaceutically acceptable carrier” as used herein may refer to compounds and compositions that are suitable for use in human or animal subjects, as for example, for therapeutic compositions administered for the treatment of a RAGE-mediated disorder or disease.

The term “pharmaceutical composition” is used herein to denote a composition that may be administered to a mammalian host, e.g., orally, parenterally, topically, by inhalation spray, intranasally, or rectally, in unit dosage formulations containing conventional non-toxic carriers, diluents, adjuvants, vehicles and the like.

The term “parenteral” as used herein, includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques.

As used herein “rejection” refers to the immune or inflammatory response on tissue that leads to destruction of cells, tissues or organs, or that leads to damage to cells, tissues, or organs. The rejected cells, tissue, or organ may be derived from the same subject that is mounting the rejection response, or may be transplanted from a different subject into the subject that is displaying rejection.

As used herein, the term “cell” refers to the structural and functional units of a mammalian living system that each comprise an independent living system. As is known in the art, cells include a nucleus, cytoplasm, intracellular organelles, and a cell wall which encloses the cell and allows the cell to be independent of other cells.

As used herein, the term “tissue” refers to an aggregate of cells that have a similar structure and function, or that work together to perform a particular function. A tissue may include a collection of similar cells and the intercellular substances surrounding the cells. Tissues include, but are not limited to, muscle tissue, nerve tissue, and bone.

As used herein an “organ” refers to a fully differentiated structural and functional unit in an animal that is specialized

for some specific function. An organ may comprise a group of tissues that perform a specific function or group of functions. Organs include, but are not limited to, the heart, lungs, brain, eye, stomach, spleen, pancreas, kidneys, liver, intestines, skin, uterus, bladder, and bone.

A “stable” formulation is one in which the RAGE fusion protein therein essentially retains its physical and chemical stability and biological activity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40° C. for 1 week to 1 month, at which time stability is measured. For example, the extent of aggregation following lyophilization and storage can be used as an indicator of RAGE fusion protein stability (see Examples herein). For example, a “stable” formulation may be one wherein less than about 10% and preferably less than about 5% of the RAGE fusion protein is present as an aggregate in the formulation. In other embodiments, an increase in aggregate formation following lyophilization and storage of the lyophilized formulation can be determined. For example, a “stable” lyophilized formulation may be one wherein the increase in aggregate in the lyophilized formulation is less than about 5% or less than about 3%, when the lyophilized formulation is incubated at 40° C. for at least one week. In other embodiments, stability of the RAGE fusion protein formulation may be measured using a biological activity assay such as a binding assay as described herein.

A “reconstituted” formulation is one which has been prepared by dissolving a lyophilized RAGE fusion protein formulation in a diluent such that the RAGE fusion protein is dispersed and/or dissolved in the reconstituted formulation. The reconstituted formulation may be suitable for administration (e.g. parenteral administration) to a patient to be treated with the fusion protein and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

By “isotonic” it is meant that the formulation of interest has an osmotic pressure from about 240 to about 340 mOsm/kg. In an embodiment, an isotonic formulation is one having an osmotic pressure that is essentially the same as human blood (285-310 mOsm/kg). Isotonicity can be measured using a vapor pressure or a freezing point depression type osmometer.

A “lyoprotectant” is a molecule which, when combined with a RAGE fusion protein, significantly prevents or reduces chemical and/or physical instability of the protein upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; a polyol such as sugar alcohols, e.g. erythritol, arabitol, xylitol, sorbitol, and mannitol; or combinations thereof. In an embodiment, the lyoprotectant may comprise a sugar. In another embodiment, the lyoprotectant may comprise a non-reducing sugar. In a further embodiment, the lyoprotectant may comprise a non-reducing sugar such as sucrose. The lyoprotectant may be added to the pre-lyophilized formulation in a “lyoprotecting amount” which means that, following lyophilization of the protein in the presence of the lyoprotecting amount of the lyoprotectant, the RAGE fusion protein essentially retains its physical and chemical stability and biological activity upon lyophilization and storage.

The “diluent” for a lyophilized formulation herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of

a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer’s solution or dextrose solution. In an embodiment, the diluent provides a reconstituted formulation suitable for injection. In another embodiment, where the diluent provides a reconstituted formulation suitable for injection, the diluent may comprise water for injection (WFI).

A “preservative” for a reconstituted formulation is a compound which can be added to the diluent or to the reconstituted formulation to essentially reduce bacterial action in the reconstituted formulation. In an embodiment, the amount of preservative may be added in an amount useful to facilitate the production of a multi-use reconstituted formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyltrimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, allyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol.

A “bulking agent” for a lyophilized formulation is a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (e.g. facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include, but are not limited to, mannitol, glycine, and xorbitol.

RAGE Fusion Proteins

Embodiments of the present invention comprise RAGE fusion proteins, methods of making such fusion proteins, and methods of use of such fusion proteins. The present invention may be embodied in a variety of ways.

For example, embodiments of the present invention provide RAGE fusion proteins comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the RAGE fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the RAGE fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence at least 90% identical thereto, or SEQ ID NO: 10 or a sequence at least 90% identical thereto, or SEQ ID NO: 47 or a sequence at least 90% identical thereto (FIG. 1).

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin domain. In one embodiment, the polypeptide comprising an immunoglobulin domain comprises at least a portion of at least one of the C_H2 or the C_H3 domains of a human IgG.

In certain embodiments, the RAGE fusion protein comprises an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine.

A RAGE protein or polypeptide may comprise full-length human RAGE protein (e.g., SEQ ID NO: 1), or a fragment of human RAGE. As used herein, a fragment of a RAGE polypeptide is at least 5 amino acids in length, may be greater than 30 amino acids in length, but is less than the full amino acid sequence. In alternate embodiments of the fusion pro-

teins, compositions, and methods of the present invention, the RAGE polypeptide may comprise a sequence that is at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neeper et al., (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence.

The RAGE fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide at least 90% identical to sRAGE, or a fragment of sRAGE. As used herein, sRAGE is the RAGE protein that does not include the transmembrane region or the cytoplasmic tail (Park et al., *Nature Med.*, 4:1025-1031 (1998)). For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (See e.g., Neeper et al., (1992)). Or, a RAGE polypeptide may comprise human sRAGE with the signal sequence removed (See e.g., SEQ ID NO: 5 or SEQ ID NO: 6, or SEQ ID NO: 45 in FIG. 1) or a portion of that amino acid sequence.

In other embodiments, the RAGE protein may comprise a RAGE V domain (see e.g., SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 46 in FIG. 1) (Neeper et al., (1992); Schmidt et al. (1997)). Or, a sequence at least 90% identical to the RAGE V domain or a fragment thereof may be used.

Or, the RAGE protein may comprise a fragment of the RAGE V domain (e.g., SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 47 in FIG. 1). In one embodiment the RAGE protein may comprise a ligand binding site. In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence at least 90% identical thereto, or SEQ ID NO: 10, or a sequence at least 90% identical thereto, or SEQ ID NO: 47, or a sequence at least 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

In another embodiment, the ligand binding site may comprise amino acids 23-53 of SEQ ID NO. 1 (FIG. 1). In another embodiment, the ligand binding site may comprise amino acids 24-52 of SEQ. ID NO: 1. In another embodiment, the ligand binding site may comprise amino acids 31-52 of SEQ IDNO: 1. In another embodiment, the ligand binding site may comprise amino acids 31-116 of SEQ ID NO: 1. In another embodiment, the ligand binding site may comprise amino acids 19-52 of SEQ ID NO: 1. For example, the ligand binding site may comprise, a RAGE V domain or a portion thereof such as the RAGE ligand binding domain (e.g., amino acids 1-118, 23-118, 24-118, 31-118, 1-116, 23-116, 24-116, 31-116, 1-54, 23-54, 24-54, 31-54, 1-53, 23-53, 24-53, or 31-53 of SEQ ID NO: 1, or fragments thereof). Or fragments of the polypeptides that functionally bind a RAGE ligand may be used. Or, a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the RAGE V domain or a fragment thereof (e.g., as described above) may be used. Further, as is known in the art, in embodiments where the N-terminus of the fusion protein is glutamine, as for example upon removal of the signal sequence comprising residues 1-23 of SEQ ID NO: 1 (e.g., Q24 for a polypeptide comprise amino acids 24-118 or SEQ ID NO: 1), the glutamine may cyclize to form pyroglutamic acid (pE).

Thus, the RAGE polypeptide used in the RAGE fusion proteins of the present invention may comprise a fragment of full length RAGE. As is known in the art, RAGE comprises three immunoglobulin-like polypeptide domains, the V domain, and the C1 and C2 domains each linked to each other

by an interdomain linker. Full-length RAGE also includes a transmembrane polypeptide and a cytoplasmic tail downstream (C-terminal) of the C2 domain, and linked to the C2 domain.

In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE. Further, as is known in the art, in embodiments where the N-terminus of the fusion protein is glutamine, (e.g., the signal sequence comprises residues 1-23), the N-terminal glutamine (Q24) may cyclize to form pyroglutamic acid (pE). Example constructs of such molecules are polypeptides having the amino acid sequences as set forth in SEQ ID NOs: 45, 46, 47, 48, 49, 50, and 51 (FIG. 1), as well as RAGE fusion proteins having the amino acid sequences as set forth in SEQ ID NOs: 56 and 57 (FIG. 4).

As recognized in the art, the C_H3 region of the RAGE fusion proteins of the present invention may have the C-terminal amino acid cleaved off through a post-translational modification when expressed in certain recombinant systems. (See e.g., Li, et al., *BioProcessing J.*, 4:23-30 (2005)). In an embodiment, the C-terminal amino acid cleaved off is lysine (K). Thus, in alternate embodiments, the RAGE fusion protein of the present invention may comprise a polypeptide having the amino acid sequence as set forth in SEQ ID NOs: 32-37, 56 and 57 without the C-terminal lysine (K).

Thus in various embodiments, the RAGE polypeptide may comprise amino acids 23-116 of human RAGE (SEQ ID NO: 7) or a sequence at least 90% identical thereto, or amino acids 24-116 of human RAGE (SEQ ID NO: 8) or a sequence at least 90% identical thereto, or amino acids 24-116 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 46) or a sequence at least 90% identical thereto, corresponding to the V domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 124-221 of human RAGE (SEQ ID NO: 11) or a sequence at least 90% identical thereto, corresponding to the C1 domain of RAGE. In another embodiment, the RAGE polypeptide may comprise amino acids 227-317 of human RAGE (SEQ ID NO: 12) or a sequence at least 90% identical thereto, corresponding to the C2 domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 23-123 of human RAGE (SEQ ID NO: 13) or a sequence at least 90% identical thereto, or amino acids 24-123 of human RAGE (SEQ ID NO: 14) or a sequence at least 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 24-123 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 48) or a sequence at least 90% identical thereto. Or, the RAGE polypeptide may comprise amino acids 23-226 of human RAGE (SEQ ID NO: 17) or a sequence at least 90% identical thereto, or amino acids 24-226 of human RAGE (SEQ ID NO: 18) or a sequence at least 90% identical thereto, corresponding to the V-domain, the C1 domain and the interdomain linker linking these two domains. Or, the RAGE polypeptide may comprise amino acids 24-226 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 50), or a sequence at least 90% identical thereto. Or, the RAGE polypeptide may comprise amino acids 23-339 of human RAGE (SEQ ID NO: 5) or a sequence at least 90% identical thereto, or 24-339 of human RAGE (SEQ ID NO: 6) or a sequence at least 90% identical thereto, corresponding to sRAGE (i.e., encoding the V, C1, and C2 domains and interdomain linkers). Or, the RAGE polypeptide may comprise amino acids 24-339 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 45) or a sequence at least 90% identical thereto. Or, fragments of each of these

sequences may be used. See FIG. 1 for the amino acid sequences of these polypeptides.

The RAGE fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the RAGE fusion protein may comprise a polypeptide derived from an immunoglobulin. In one embodiment, the immunoglobulin polypeptide may comprise an immunoglobulin heavy chain or a portion (i.e., fragment) thereof. For example, the heavy chain fragment may comprise a polypeptide derived from the Fc fragment of an immunoglobulin, wherein the Fc fragment comprises the heavy chain hinge polypeptide, and C_{H2} and C_{H3} domains of the immunoglobulin heavy chain as a monomer. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ϵ), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ 1), IgG2 (γ 2), IgG3 (γ 3), IgG4 (γ 4), IgA1 (α 1), IgA2 (α 2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_{H2} and C_{H3} domains of a human IgG1 or portions of either, or both, of these domains. As an example embodiment, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 40 (FIG. 1) or a portion thereof. In one embodiment, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or a fragment thereof. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41 (FIG. 1). The immunoglobulin sequence in SEQ ID NO: 38 or SEQ ID NO: 40 may also be encoded by SEQ ID NO: 52 or SEQ ID NO: 53 (FIG. 1), where silent base changes for the codons that encode for proline (CCG to CCC) and glycine (GGT to GGG) at the C-terminus of the sequence remove a cryptic RNA splice site near the terminal codon (i.e., nucleotides 622-627 of SEQ ID NO: 39 are modified to generate SEQ ID NO: 52 or nucleotides 652-657 of SEQ ID NO: 41 are modified to generate SEQ ID NO: 53).

The hinge region of the Fc portion of the immunoglobulin chain may be proinflammatory in vivo. Thus, in one embodiment, the RAGE fusion protein of the present invention comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

Thus in certain embodiments, the RAGE fusion protein may comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_{H2} domain of an immunoglobulin, or a fragment or portion of the C_{H2} domain of an immunoglobulin. In one embodiment, the C_{H2} domain, or a fragment thereof comprises SEQ ID NO: 42 (FIG. 1). In an embodiment, the fragment of SEQ ID NO: 42 comprises SEQ ID NO: 42 with the first ten amino acids comprising at least a portion of the Fc hinge region removed. In one embodiment, the RAGE polypeptide may comprise a ligand binding site. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence at least 90% identical thereto, or SEQ ID NO: 10 or a sequence at least 90% identical thereto, or SEQ ID NO: 47, or a sequence at least 90% identical thereto.

The RAGE polypeptide used in the RAGE fusion proteins of the present invention may comprise a RAGE immunoglobulin domain. Additionally or alternatively, the fragment of RAGE may comprise an interdomain linker. Or, the RAGE polypeptide may comprise a RAGE immunoglobulin domain linked to an upstream (i.e., closer to the N-terminus) or down-

stream (i.e., closer to the C-terminus) interdomain linker. In yet another embodiment, the RAGE polypeptide may comprise two (or more) RAGE immunoglobulin domains each linked to each other by an interdomain linker. The RAGE polypeptide may further comprise multiple RAGE immunoglobulin domains linked to each other by one or more interdomain linkers and having a terminal interdomain linker attached to the N-terminal RAGE immunoglobulin domain and/or the C-terminal immunoglobulin domain. Additional combinations of RAGE immunoglobulin domains and interdomain linkers are within the scope of the present invention.

In one embodiment, the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_{H2} domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_{H2} domain of an immunoglobulin may comprise the C_{H2} and C_{H3} domains of a human IgG1 or a portion of either, or both, of these domains. As example embodiments, the polypeptide comprising the C_{H2} and C_{H3} domains, or a portion thereof, of a human IgG1 may comprise SEQ ID NO: 40 or a portion thereof. In one embodiment, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or a fragment thereof. Or, the human IgG1 may comprise SEQ ID NO: 38 or SEQ ID NO: 40 with the terminal lysine (K) removed.

As described above, the RAGE fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE polypeptide domain may comprise a fragment of full-length RAGE protein. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence at least 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence at least 90% identical thereto, or amino acids 24-136 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 49), or a sequence at least 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence at least 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence at least 90% identical thereto, or amino acids 24-251 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 51), or a sequence at least 90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1.

For example, in one embodiment, the RAGE fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The RAGE fusion protein may comprise a first RAGE immunoglobulin domain and a first RAGE interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the second RAGE immunoglobulin domain, and the C-terminal amino acid of the RAGE second

interdomain linker is directly linked to the N-terminal amino acid of the C_H2 immunoglobulin domain. In alternate embodiments, the four domain RAGE fusion protein is encoded by SEQ ID NO: 30 or SEQ ID NO: 54 (FIG. 2). In one embodiment, a four domain RAGE fusion protein may comprise SEQ ID NO: 32 (FIG. 4). In alternate embodiments, a four domain RAGE fusion protein comprises SEQ ID NO: 33, SEQ ID NO: 34, or SEQ ID NO: 56 (FIG. 4).

Alternatively, a three domain RAGE fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the RAGE fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of a C_H2 immunoglobulin domain or a portion of a C_H2 immunoglobulin domain. In alternate embodiments, the three domain RAGE fusion protein is encoded by SEQ ID NO: 31 or SEQ ID NO: 55 (FIG. 3). In one embodiment, a three domain RAGE fusion protein may comprise SEQ ID NO: 35 (FIG. 5). In alternate embodiments, a three domain RAGE fusion protein may comprise SEQ ID NO: 36, SEQ ID NO: 37, or SEQ ID NO: 57 (FIG. 5).

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, an interdomain linker comprising several amino acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a peptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. In alternate embodiments, the linker may comprise a peptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 22 or SEQ ID NO: 24.

Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342. In alternate embodiments, the linker may comprise a peptide that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 44.

Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than about 5%, more typically less than about 1%) in an encoded sequence are conservatively modified

variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following example groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
 - 2) Aspartic acid (D), Glutamic acid (E);
 - 3) Asparagine (N), Glutamine (Q);
 - 4) Arginine (R), Lysine (K);
 - 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

A conservative substitution is a substitution in which the substituting amino acid (naturally occurring or modified) is structurally related to the amino acid being substituted, i.e., has about the same size and electronic properties as the amino acid being substituted. Thus, the substituting amino acid would have the same or a similar functional group in the side chain as the original amino acid. A “conservative substitution” also refers to utilizing a substituting amino acid which is identical to the amino acid being substituted except that a functional group in the side chain is protected with a suitable protecting group.

As is known in the art, amino acids may become chemically modified from their natural structure, either by enzymatic or non-enzymatic reaction mechanisms. For example, in one embodiment, an N-terminal glutamic acid or glutamine may cyclize, with loss of water, to form pyroglutamic acid (pyroE or pE) (Chelius et al., *Anal. Chem*, 78: 2370-2376 (2006) and Burstein et al., *Proc. National Acad. Sci.*, 73:2604-2608 (1976)). Alternatively, a fusion protein having an N-terminal pyroglutamic acid could potentially be accessed through a nucleic acid sequence encoding for glutamic acid at the position in the protein that via post-translational processing becomes the N-terminus (e.g., where residue 24 of SEQ ID NO: 1 is glutamate rather than a glutamine).

Methods of Producing RAGE Fusion Proteins

The present invention also comprises a method to make a RAGE fusion protein. Thus, in one embodiment, the present invention comprises a method of making a RAGE fusion protein comprising the step of covalently linking a RAGE polypeptide linked to a second, non-RAGE polypeptide wherein the RAGE polypeptide comprises a RAGE ligand binding site. For example, the linked RAGE polypeptide and the second, non-RAGE polypeptide may be encoded by a recombinant DNA construct. The method may further comprise the step of incorporating the DNA construct into an expression vector. Also, the method may comprise the step of inserting the expression vector into a host cell.

For example, embodiments of the present invention provide RAGE fusion proteins comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the RAGE fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the RAGE fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence at least 90% identical thereto, or SEQ ID NO: 10 or a sequence at least 90% identical thereto, or SEQ ID NO: 47, or a sequence at least 90% identical thereto.

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin domain. In one embodiment, the polypeptide comprising an

immunoglobulin domain comprises at least a portion of at least one of the C_{H2} or the C_{H3} domains of a human IgG.

Thus, embodiments of the present invention may comprise isolated DNA molecules that encode the RAGE fusion proteins of the present invention. In certain embodiments, the DNA molecules encode for a RAGE fusion protein comprising an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine. Thus, in certain embodiments, the present invention may comprise a DNA molecule having the sequence as set forth in SEQ ID NO: 54 or SEQ ID NO: 55, or a sequence at least 90% identical thereto.

The RAGE fusion protein may be engineered by recombinant DNA techniques. For example, in one embodiment, the present invention may comprise an isolated nucleic acid sequence comprising, complementary to, or having significant identity with, a polynucleotide sequence that encodes for a RAGE polypeptide linked to a second, non-RAGE polypeptide. In an embodiment, the RAGE polypeptide may comprise a RAGE ligand binding site.

The RAGE protein or polypeptide may comprise full-length human RAGE (e.g., SEQ ID NO: 1), or a fragment of human RAGE. In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE (SEQ ID NO: 1). In alternate embodiments, the RAGE polypeptide may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Nepper et al., (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence. The RAGE fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide at least 90% identical to sRAGE, or a fragment of sRAGE. For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Nepper et al., (1992)). Or, the human RAGE may comprise sRAGE with the signal sequence removed (See e.g., SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 45 in FIG. 1) or a portion of that amino acid sequence. In other embodiments, the RAGE protein may comprise a V domain (See e.g., SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 46 in FIG. 1). Or, a sequence at least 90% identical to the V domain or a fragment thereof may be used. Or, the RAGE protein may comprise a fragment of RAGE comprising a portion of the V domain (See e.g., SEQ ID NO: 9, SEQ ID NO: 10 or SEQ ID NO: 47 in FIG. 1). In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence at least 90% identical thereto, or SEQ ID NO: 10, or a sequence at least 90% identical thereto, or SEQ ID NO: 47, or a sequence at least 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

In an embodiment, the nucleic acid sequence comprises SEQ ID NO: 25 to encode amino acids 1-118 of human RAGE or a fragment thereof. For example, a sequence comprising nucleotides 1-348 of SEQ ID NO: 25 may be used to encode amino acids 1-116 of human RAGE. Or, the nucleic

acid may comprise SEQ ID NO: 26 to encode amino acids 1-123 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 27 to encode amino acids 1-136 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 28 to encode amino acids 1-230 of human RAGE. Or, the nucleic acid may comprise SEQ ID NO: 29 to encode amino acids 1-251 of human RAGE. Or fragments of these nucleic acid sequences may be used to encode RAGE polypeptide fragments.

The RAGE fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the RAGE fusion protein may comprise a polypeptide derived from an immunoglobulin. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ϵ), or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ 1), IgG2 (γ 2), IgG3 (γ 3), IgG4 (γ 4), IgA1 (α 1), IgA2 (α 2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_{H2} and C_{H3} domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40. In one embodiment, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or a fragment thereof. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41. In alternate embodiments, the immunoglobulin sequence in SEQ ID NO: 38 or SEQ ID NO: 40 may also be encoded by SEQ ID NO: 52 or SEQ ID NO: 53, respectively.

The hinge region of the Fc portion of the immunoglobulin chain may be proinflammatory in vivo. Thus, the RAGE fusion protein of the present invention may comprise an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

Thus, in one embodiment, the present invention comprises a method of making a RAGE fusion protein comprising the step of covalently linking a RAGE polypeptide to a polypeptide comprising a C_{H2} domain of an immunoglobulin or a portion of a C_{H2} domain of an immunoglobulin. In one embodiment, the RAGE fusion protein may comprise a RAGE ligand binding site. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence at least 90% identical thereto, or SEQ ID NO: 10 or a sequence at least 90% identical thereto, or SEQ ID NO: 47, or a sequence at least 90% identical thereto.

In one embodiment, the RAGE fusion protein may be encoded by a recombinant DNA construct. The method may comprise the step of incorporating the DNA construct into an expression vector. Also, the method may comprise transfecting the expression vector into a host cell. Thus, embodiments of the present invention also comprise expression vectors encoding DNA molecules that encode the RAGE fusion proteins of the present invention. In certain embodiments, the DNA molecules encode for a RAGE fusion protein comprising an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine.

Yet other embodiments of the present invention also comprise cells transfected with an expression vector encoding DNA molecules that encode the RAGE fusion proteins of the present invention. In certain embodiments, the DNA molecules encode for a RAGE fusion protein comprising an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine.

For example, in one embodiment, the present invention comprises a nucleic acid encoding a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. In one embodiment, the C_H2 domain, or a fragment thereof, comprises SEQ ID NO: 42. In an embodiment, the fragment of SEQ ID NO: 42 comprises SEQ ID NO: 42 with the first ten amino acids removed. The second polypeptide may comprise the C_H2 and C_H3 domains of a human IgG1. As example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 may comprise SEQ ID NO: 38 or SEQ ID NO: 40. In one embodiment, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or a fragment thereof. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41. The immunoglobulin sequence in SEQ ID NO: 38 or SEQ ID NO: 40 may also be encoded by SEQ ID NO: 52 or SEQ ID NO: 53, where silent base changes for the codons that encode for proline (CCG to CCC) and glycine (GGT to GGG) at the C-terminus of the sequence remove a cryptic RNA splice site near the terminal codon (i.e., nucleotides 622-627 of SEQ ID NO: 39 are modified to generate SEQ ID NO: 52 or nucleotides 652-657 of SEQ ID NO: 41 are modified to generate SEQ ID NO: 53).

In one embodiment, the RAGE polypeptide may comprise a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof, may comprise a polypeptide comprising the C_H2 and C_H3 domains of a human IgG1 or a portion of both, or either, of these domains. As example embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or SEQ ID NO: 40. In one embodiment, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or a fragment thereof. In certain embodiments, the polypeptide comprising the C_H2 and C_H3 domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 38 or SEQ ID NO: 40 with the C-terminal lysine (K) removed.

The RAGE fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE immunoglobulin domain may comprise a fragment of a full-length RAGE protein. For example, in one embodiment, the RAGE fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The RAGE fusion protein may comprise a first

RAGE immunoglobulin domain and a first interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the RAGE second immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence at least 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence at least 90% identical thereto, or amino acids 24-251 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 51) or a sequence at least 90% identical thereto, corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 30 or a fragment thereof may encode for a four domain RAGE fusion protein (FIG. 2A). In another embodiment, nucleic acid construct comprising SEQ ID NO: 54 (FIG. 2B) may encode for a four domain RAGE fusion protein, where silent base changes for the codons that encode for proline (CCG to CCC) and glycine (GGT to GGG) at the C-terminus of the sequence are entered to remove a cryptic RNA splice site near the terminal codon (i.e., at nucleotides 1375-1380 of SEQ ID NO: 30 are modified to generate SEQ ID NO: 54).

Alternatively, a three domain RAGE fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the RAGE fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising a C_H2 immunoglobulin domain or a fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence at least 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence at least 90% identical thereto, or amino acids 24-136 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 49) or a sequence at least 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker (FIG. 1). In one embodiment, a nucleic acid construct comprising SEQ ID NO: 31 or a fragment thereof may encode for a three domain RAGE fusion protein (FIG. 3A). In another embodiment, nucleic acid construct comprising SEQ ID NO: 55 may encode for a three domain RAGE fusion protein, where silent base changes for the codons that encode for proline (CCG to CCC) and glycine (GGT to GGG) at the C-terminus of the sequence remove a cryptic RNA splice site near the terminal codon (i.e., nucleotides 1030-1035 of SEQ ID NO: 31 are modified to generate SEQ ID NO: 55) (FIG. 3B).

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having

additional portions of the natural RAGE sequence. For example, an interdomain linker comprising several amino acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise a peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. In alternate embodiments, the linker may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 22 or SEQ ID NO: 24.

Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342. In alternate embodiments, the linker may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 44.

The method may further comprise the step of incorporating the DNA construct into an expression vector. Thus, in an embodiment, the present invention comprises an expression vector that encodes for a RAGE fusion protein comprising a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin. In an embodiment, the RAGE polypeptide comprise constructs, such as those described herein, having a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof. For example, the expression vector used to transfect the cells may comprise the nucleic acid sequence SEQ ID NO: 30, or a fragment thereof, SEQ ID NO: 54, or a fragment thereof, SEQ ID NO: 31, or a fragment thereof, or SEQ ID NO: 55, or a fragment thereof.

The method may further comprise the step of transfecting a cell with the expression vector of the present invention. Thus, in an embodiment, the present invention comprises a cell transfected with the expression vector that expressed the RAGE fusion protein of the present invention, such that the cell expresses a RAGE fusion protein comprising a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin. In an embodiment, the RAGE polypeptide comprise constructs, such as those described herein, having a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly

linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof. For example, the expression vector may comprise the nucleic acid sequence SEQ ID NO: 30, or a fragment thereof, SEQ ID NO: 54, or a fragment thereof, SEQ ID NO: 31, or a fragment thereof, or SEQ ID NO: 55, or a fragment thereof.

For example, plasmids may be constructed to express RAGE-IgG fusion proteins by fusing different lengths of a 5' cDNA sequence of human RAGE with a 3' cDNA sequence of human IgG1 Fc ($\gamma 1$). The expression cassette sequences may be inserted into an expression vector such as pcDNA3.1 expression vector (Invitrogen, CA) using standard recombinant techniques.

Also, the method may comprise transfecting the expression vector into a host cell. RAGE fusion proteins may be expressed in mammalian expression systems, including systems in which the expression constructs are introduced into the mammalian cells using virus such as retrovirus or adenovirus. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, inter alia, Chinese hamster ovary (CHO) cells, NS0, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines. Cell lines may be selected through determining which cell lines have high expression levels of a RAGE fusion protein. Other cell lines that may be used are insect cell lines, such as Sf9 cells. Plant host cells include, e.g., *Nicotiana*, *Arabidopsis*, duckweed, corn, wheat, potato, etc. Bacterial host cells include *E. coli* and *Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*. When recombinant expression vectors encoding RAGE fusion protein genes are introduced into mammalian host cells, the RAGE fusion proteins are produced by culturing the host cells for a period of time sufficient to allow for expression of the RAGE fusion protein in the host cells or secretion of the RAGE fusion protein into the culture medium in which the host cells are grown. RAGE fusion proteins may be recovered from the culture medium using standard protein purification methods.

Nucleic acid molecules encoding RAGE fusion proteins and expression vectors comprising these nucleic acid molecules may be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell. Transformation may be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming plant cells are well known in the art, including, e.g., *Agrobacterium*-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

An expression vector may also be delivered to an expression system using DNA biolistics, wherein the plasmid is precipitated onto microscopic particles, preferably gold, and the particles are propelled into a target cell or expression system. DNA biolistics techniques are well-known the art and devices, e.g., a "gene gun", are commercially available for delivery of the microparticles in to a cell (e.g., Helios Gene

Gun, Bio-Rad Labs., Hercules, Calif.) and into the skin (PMED Device, PowderMed Ltd., Oxford, UK).

Expression of RAGE fusion proteins from production cell lines may be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) and the plasma-encoded neomycin resistance system are common approaches for enhancing expression under certain conditions.

RAGE fusion proteins expressed by different cell lines may have different glycosylation patterns from each other. However, all RAGE fusion proteins encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation of the RAGE fusion protein.

In one embodiment, a recombinant expression vector may be transfected into Chinese Hamster Ovary cells (CHO) and expression optimized. In alternate embodiments, the cells may produce 0.1 to 20 grams/liter, or 0.5 to 10 grams/liter, or about 1-2 grams/liter.

As is known in the art, such nucleic acid constructs may be modified by mutation, as for example, by PCR amplification of a nucleic acid template with primers comprising the mutation of interest. In this way, polypeptides comprising varying affinity for RAGE ligands may be designed. In one embodiment, the mutated sequences may be 90% or more identical to the starting DNA. As such, variants may include nucleotide sequences that hybridize under stringent conditions (i.e., equivalent to about 20-27° C. below the melting temperature (T_M) of the DNA duplex in 1 molar salt).

The coding sequence may be expressed by transfecting the expression vector into an appropriate host. For example, the recombinant vectors may be stably transfected into Chinese Hamster Ovary (CHO) cells, and cells expressing the RAGE fusion protein selected and cloned. In an embodiment, cells expressing the recombinant construct are selected for plasmid-encoded neomycin resistance by applying antibiotic G418. Individual clones may be selected and clones expressing high levels of recombinant protein as detected by Western Blot analysis of the cell supernatant may be expanded, and the gene product purified by affinity chromatography using Protein A columns.

Sample embodiments of recombinant nucleic acids that encode the RAGE fusion proteins of the present invention are shown in FIGS. 2 and 3. For example, as described above, the RAGE fusion protein produced by the recombinant DNA construct may comprise a RAGE polypeptide linked to a second, non-RAGE polypeptide. The RAGE fusion protein may comprise two domains derived from RAGE protein and two domains derived from an immunoglobulin. Example nucleic acid constructs encoding a RAGE fusion protein, TTP-4000 (TT4), having this type of structure is shown in FIG. 2 (SEQ ID NO: 30 and SEQ ID NO: 54). As shown for SEQ ID NO: 30 and SEQ ID NO: 54, coding sequence 1-753 (highlighted in bold) encodes the RAGE N-terminal protein sequence whereas the sequence from 754-1386 encodes the IgG Fc protein sequence without the hinge.

When derived from SEQ ID NO: 30 or SEQ ID NO: 54, or a sequence at least 90% identical thereto, the RAGE fusion protein may comprise the four domain amino acid sequence of SEQ ID NO: 32, or the polypeptide with the signal sequence removed (see e.g., SEQ ID NO: 33, SEQ ID NO: 34 or SEQ ID NO: 56 in FIG. 4). In SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 or SEQ ID NO: 56, the RAGE amino acid sequence is highlighted with bold font. The immunoglobulin sequence is the C_H2 and C_H3 immunoglobulin domains of IgG without the hinge region.

FIG. 6 shows a comparison of the polypeptide domains found in RAGE and IgG (FIG. 6A) and the domain structure of the RAGE fusion proteins TTP-3000 and TTP-4000. As shown in FIG. 6B, the first 251 amino acids of the full-length TTP-4000 RAGE fusion protein contains as the RAGE polypeptide sequence a signal sequence comprising amino acids 1-22/23, the V immunoglobulin domain (including the ligand binding site) comprising amino acids 23/24-116, an interdomain linker comprising amino acids 117 to 123, a second immunoglobulin domain (C1) comprising amino acids 124-221, and a downstream interdomain linker comprising amino acids 222-251.

In an embodiment, the RAGE fusion protein may not necessarily comprise the second RAGE immunoglobulin domain. For example, the RAGE fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. Example nucleic acid constructs encoding this type of RAGE fusion protein is shown in FIG. 3 (SEQ ID NO: 31 and SEQ ID NO: 55). As shown in SEQ ID NO: 31 and SEQ ID NO: 55, the coding sequence from nucleotides 1 to 408 (highlighted in bold) encodes the RAGE N-terminal protein sequence, whereas the sequence from 409-1041 codes the IgG1 Fc (γ1) protein sequence.

When derived from SEQ ID NO: 31 or SEQ ID NO: 55, or a sequence at least 90% identical thereto, the RAGE fusion protein may comprise the three domain amino acid sequence of SEQ ID NO: 35, or the polypeptide with the signal sequence removed (see e.g., SEQ ID NO: 36, SEQ ID NO: 37 or SEQ ID NO: 57 in FIG. 5). In SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37 or SEQ ID NO: 57 in FIG. 5, the RAGE amino acid sequence is highlighted with bold font. As shown in FIG. 6B, the first 136 amino acids of the full-length TTP-3000 RAGE fusion protein contains as the RAGE polypeptide a signal sequence comprising amino acids 1-22/23, the V immunoglobulin domain (including the ligand binding site) comprising amino acids 23/24-116, and an interdomain linker comprising amino acids 117 to 136. The sequence from 137 to 346 includes the C_H2 and C_H3 immunoglobulin domains of IgG without the hinge region.

The RAGE fusion proteins of the present invention may comprise improved in vivo stability over RAGE polypeptides not comprising a second polypeptide. The RAGE fusion protein may be further modified to increase stability, efficacy, potency and bioavailability. Thus, the RAGE fusion proteins of the present invention may be modified by post-translational processing or by chemical modification. For example, the RAGE fusion protein may be synthetically prepared to include L-, D-, or unnatural amino acids, alpha-disubstituted amino acids, or N-alkyl amino acids. Additionally, proteins may be modified by acetylation, acylation, ADP-ribosylation, amidation, attachment of lipids such as phosphatidylinositol, formation of disulfide bonds, and the like. Furthermore, polyethylene glycol can be added to increase the biological stability of the RAGE fusion protein.

Binding of RAGE Antagonists to RAGE Fusion Proteins

The RAGE fusion proteins of the present invention may comprise a number of applications. For example, the RAGE fusion protein of the present invention may be used in a binding assay to identify RAGE ligands, such as RAGE agonists, antagonists, or modulators.

For example, in one embodiment, the present invention provides a method for detection of RAGE modulators comprising: (a) providing a RAGE fusion protein comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide, where the RAGE polypeptide comprises a ligand binding site; (b) mixing a compound of interest and a ligand

having a known binding affinity for RAGE with the RAGE fusion protein; and (c) measuring binding of the known RAGE ligand to the RAGE fusion protein in the presence of the compound of interest. In an embodiment, the ligand binding site comprises the most N-terminal domain of the RAGE fusion protein.

The RAGE fusion proteins may also provide kits for the detection of RAGE modulators. For example, in one embodiment, a kit of the present invention may comprise (a) a compound having known binding affinity to RAGE as a positive control; (b) a RAGE fusion protein comprising a RAGE polypeptide linked to a second, non-RAGE polypeptide, wherein the RAGE polypeptide comprises a RAGE ligand binding site; and (c) instructions for use. In an embodiment, the ligand binding site comprises the most N-terminal domain of the RAGE fusion protein.

For example, the RAGE fusion protein may be used in a binding assay to identify potential RAGE ligands. In one example embodiment of such a binding assay, a known RAGE ligand may be coated onto a solid substrate (e.g., Maxisorb plates) at a concentration of about 5 micrograms per well, where each well contains a total volume of about 100 microliters (4). The plates may be incubated at 4° C. overnight to allow the ligand to absorb or bind to the substrate. Alternatively, shorter incubation periods at higher temperature (e.g., room temperature) may be used. After a period of time to allow for the ligand to bind to the substrate, the assay wells may be aspirated and a blocking buffer (e.g., 1% BSA in 50 mM imidazole buffer, pH 7.2) may be added to block nonspecific binding. For example, blocking buffer may be added to the plates for 1 hour at room temperature. The plates may then be aspirated and/or washed with a wash buffer. In one embodiment, a buffer comprising 20 mM Imidazole, 150 mM NaCl, 0.05% Tween-20, 5 mM CaCl₂ and 5mM MgCl₂, pH 7.2 may be used as a wash buffer. The RAGE fusion protein may then be added at increasing dilutions to the assay wells. The RAGE fusion protein may then be allowed to incubate with the immobilized ligand in the assay well such that binding can attain equilibrium. In one embodiment, the RAGE fusion protein is allowed to incubate with the immobilized ligand for about one hour at 37° C. In alternate embodiments, longer incubation periods at lower temperatures may be used. After the RAGE fusion protein and immobilized ligand have been incubated, the plate may be washed to remove any unbound RAGE fusion protein. The RAGE fusion protein bound to the immobilized ligand may be detected in a variety of ways. In one embodiment, detection employs an ELISA. Thus, in one embodiment, an immunodetection complex containing a monoclonal mouse anti-human IgG1, biotinylated goat anti-mouse IgG, and an avidin linked alkaline phosphatase may be added to the RAGE fusion protein immobilized in the assay well. The immunodetection complex may be allowed to bind to the immobilized RAGE fusion protein such that binding between the RAGE fusion protein and the immunodetection complex attains equilibrium. For example, the complex may be allowed to bind to the RAGE fusion protein for one hour at room temperature. At that point, any unbound complex may be removed by washing the assay well with wash buffer. The bound complex may be detected by adding the alkaline phosphatase substrate, para-nitrophenylphosphate (PNPP), and measuring conversion of PNPP to para-nitrophenol (PNP) as an increase in absorbance at 405 nm.

In an embodiment, RAGE ligand binds to the RAGE fusion protein with nanomolar (nM) or micromolar (μM) affinity. An experiment illustrating binding of RAGE ligands to RAGE fusion proteins of the present invention is shown in FIG. 7.

Solutions of TTP-3000 (TT3) and TTP-4000 (TT4) having initial concentrations of 1.082 mg/mL, and 370 μg/mL, respectively, were prepared. As shown FIG. 7, at various dilutions, the RAGE fusion proteins TTP-3000 and TTP-4000 are able to bind to immobilized RAGE ligands Amyloid-beta (Abeta) (Amyloid Beta (1-40) from Biosource), S100b (S100), and amphotericin (Ampho), resulting in an increase in absorbance. In the absence of ligand (i.e., coating with only BSA) there was no increase in absorbance.

The binding assay of the present invention may be used to quantify ligand binding to RAGE. In alternate embodiments, RAGE ligands may bind to the RAGE fusion protein of the present invention with binding affinities ranging from 0.1 to 1000 nanomolar (nM), or from 1 to 500 nM, or from 10 to 80 nM.

The RAGE fusion protein of the present invention may also be used to identify compounds having the ability to bind to RAGE. As shown in FIGS. 8 and 9, respectively, a RAGE ligand may be assayed for its ability to compete with immobilized amyloid beta for binding to TTP-4000 (TT4) or TTP-3000 (TT3), RAGE fusion proteins. Thus, it may be seen that a RAGE ligand at a final assay concentration (FAC) of 10 μM can displace binding of RAGE fusion protein to amyloid-beta at concentrations of 1:3, 1:10, 1:30, and 1:100 of the initial TTP-4000 solution (FIG. 8) or TTP-3000 (FIG. 9).

Modulation of Cellular Effectors

Embodiments of the RAGE fusion proteins of the present invention may be used to modulate a biological response mediated by RAGE. For example, the RAGE fusion proteins may be designed to modulate RAGE-induced increases in gene expression. Thus, in an embodiment, RAGE fusion proteins of the present invention may be used to modulate the function of biological enzymes. For example, the interaction between RAGE and its ligands may generate oxidative stress and activation of NF-κB, and NF-κB regulated genes, such as the cytokines IL-1β, TNF-α, and the like. In addition, several other regulatory pathways, such as those involving p21ras, MAP kinases, ERK1, and ERK2, have been shown to be activated by binding of AGEs and other ligands to RAGE.

Use of the RAGE fusion proteins of the present invention to modulate expression of the cellular effector TNF-α is shown in FIG. 10. THP-1 myeloid cells may be cultured in RPMI-1640 media supplemented with 10% FBS and induced to secrete TNF-α via stimulation of RAGE with S100b. When such stimulation occurs in the presence of a RAGE fusion protein, induction of TNF-α by S100b binding to RAGE may be inhibited. Thus, as shown in FIG. 10, addition of 10 μg TTP-3000 (TT3) or TTP-4000 (TT4) RAGE fusion protein reduces S100b induction of TNF-α by about 50% to 75%. RAGE fusion protein TTP-4000 may be at least as effective in blocking S100b induction of TNF-α as is sRAGE (FIG. 10). Specificity of the inhibition for the RAGE sequences of TTP-4000 and TTP-3000 is shown by the experiment in which IgG alone was added to S100b stimulated cells. Addition of IgG and S100b to the assay shows the same levels of TNF-α as S100b alone.

In another cell-based assay, the ability of TTP-4000 to prevent the RAGE ligand HMGB1 from interacting with RAGE and other HMGB1 receptors was evaluated. Unlike anti-RAGE antibodies that bind to RAGE and to prevent the interaction of a RAGE ligand with RAGE, TTP-4000 may block the interaction of a RAGE ligand with RAGE by binding to the RAGE ligand. HMGB1 has been reported to be a ligand for RAGE and the Toll-Like Receptors 2 and 4 (Park et al., *J Biol Chem.*, 2004; 279(9):7370-7). All three of these

receptors (RAGE, Toll-like receptor 2, and Toll-like receptor 4) are expressed on THP-1 cells (Parker, et al., *J Immunol.*, 2004,172(8):4977-86.).

In this experiment, THP-1 cells were stimulated to produce TNF- α by HMGB1 (50 mg/mL) in the presence or absence of either TTP4000 or anti-RAGE antibodies. Under the conditions used in the assay, HMGB1 should be the only inducer of TNF- α . The results in FIG. 11 demonstrate that the anti-RAGE antibody and RAGE fusion protein TTP-4000 block HMGB1 from interacting with RAGE expressed on the THP-1 cells, and that TTP-4000 inhibits HMGB1-induced TNF- α production to a greater extent than does the anti-RAGE antibody. Thus, the data indicate that TTP-4000 may inhibit HMGB1 activity to a greater extent than anti-RAGE antibody by inhibiting HMGB1 from interacting with Toll-like receptors 2 and 4, as well as RAGE present on THP-1 cells.

Physiological Characteristics of RAGE Fusion Proteins

While sRAGE can have a therapeutic benefit in the modulation of RAGE-mediated diseases, human sRAGE may have limitations as a stand-alone therapeutic based on the relatively short half-life of sRAGE in plasma. For example, whereas rodent sRAGE has a half-life in normal and diabetic rats of approximately 20 hours, human sRAGE has a half-life of less than 2 hours when assessed by retention of immunoreactivity sRAGE (Renard et al., *J. Pharmacol. Exp. Ther.*, 290:1458-1466 (1999)).

To generate a RAGE therapeutic that has similar binding characteristics as sRAGE, but a more stable pharmacokinetic profile, a RAGE fusion protein comprising a RAGE ligand binding site linked to one or more human immunoglobulin domains may be used. As is known in the art, the immunoglobulin domains may include the Fc portion of the immunoglobulin heavy chain.

The immunoglobulin Fc portion may confer several attributes to a RAGE fusion protein. For example, the Fc fusion protein may increase the serum half-life of such fusion proteins, often from hours to several days. The increase in pharmacokinetic stability is generally a result of the interaction of the linker between C_H2 and C_H3 regions of the Fc fragment with the FcRn receptor (Wines et al., *J. Immunol.*, 164:5313-5318 (2000)).

Although fusion proteins comprising an immunoglobulin Fc polypeptide may provide the advantage of increased stability, immunoglobulin fusion proteins may elicit an inflammatory response when introduced into a host. The inflammatory response may be due, in large part, to the Fc portion of the immunoglobulin of the fusion protein. The proinflammatory response may be a desirable feature if the target is expressed on a diseased cell type that needs to be eliminated (e.g., a cancer cell, an or a population of lymphocytes causing an autoimmune disease). The proinflammatory response may be a neutral feature if the target is a soluble protein, as most soluble proteins do not activate immunoglobulins. However, the proinflammatory response may be a negative feature if the target is expressed on cell types whose destruction would lead to untoward side-effects. Also, the proinflammatory response may be a negative feature if an inflammatory cascade is established at the site of a fusion protein binding to a tissue target, since many mediators of inflammation may be detrimental to surrounding tissue, and/or may cause systemic effects.

The primary proinflammatory site on immunoglobulin Fc fragments resides on the hinge region between the C_H1 and C_H2. This hinge region interacts with the FcR1-3 on various leukocytes and trigger these cells to attack the target. (Wines et al., *J. Immunol.*, 164:5313-5318 (2000)).

As therapeutics for RAGE-mediated diseases, RAGE fusion proteins may not require the generation of an inflammatory response. Thus, embodiments of the RAGE fusion proteins of the present invention may comprise a RAGE fusion protein comprising a RAGE polypeptide linked to an immunoglobulin domain(s) where the Fc hinge region from the immunoglobulin is removed and replaced with a RAGE polypeptide. In this way, interaction between the RAGE fusion protein and Fc receptors on inflammatory cells may be minimized. It may be important, however, to maintain proper stacking and other three-dimensional structural interactions between the various immunoglobulin domains of the RAGE fusion protein. Thus, embodiments of the RAGE fusion proteins of the present invention may substitute the biologically inert, but structurally similar RAGE interdomain linker that separates the V and C1 domains of RAGE, or the linker that separates the C1 and C2 domains of RAGE, in lieu of the normal hinge region of the immunoglobulin heavy chain. Thus, the RAGE polypeptide of the RAGE fusion protein may comprise an interdomain linker sequence that is naturally found downstream of a RAGE immunoglobulin domain to form a RAGE immunoglobulin domain/linker fragment. In this way, the three dimensional interactions between the immunoglobulin domains contributed by either RAGE or the immunoglobulin may be maintained.

In an embodiment, a RAGE fusion protein of the present invention may comprise a substantial increase in pharmacokinetic stability as compared to sRAGE. For example, FIG. 12 shows that once the RAGE fusion protein TTP-4000 has saturated its ligands, it may retain a half-life of greater than 300 hours. This may be contrasted with the half-life for sRAGE of only a few hours in human plasma.

Thus, in an embodiment, the RAGE fusion proteins of the present invention may be used to antagonize binding of physiological ligands to RAGE as a means to treat RAGE-mediated diseases without generating an unacceptable amount of inflammation. The RAGE fusion proteins of the present invention may exhibit a substantial decrease in generating a proinflammatory response as compared to IgG. For example, as shown in FIG. 13, the RAGE fusion protein TTP-4000 does not stimulate TNF- α release from cells under conditions where human IgG stimulation of TNF- α release is detected.

Treatment of Disease with RAGE Fusion Proteins

The present invention may also comprise methods for the treatment of RAGE-mediated disorder in a human subject. In an embodiment, the method may comprise administering to a subject a RAGE fusion protein comprising a RAGE polypeptide comprising a RAGE ligand binding site linked to a second, non-RAGE polypeptide.

In certain embodiments, the RAGE formulation comprises a lyophilized RAGE fusion protein. In certain embodiments, the present invention may comprise methods of treating a RAGE-mediated disorder in a subject comprising administering to a subject a therapeutically effective amount of a reconstituted formulation comprising a RAGE fusion protein, a lyoprotectant, and a buffer.

Any of the embodiments of the RAGE fusion proteins described herein may be used for treatment of disease in the therapeutic compositions and formulations of the present invention. Thus, the RAGE fusion protein may comprise a sequence derived from a RAGE ligand binding site linked to an immunoglobulin polypeptide. Embodiments of the RAGE fusion protein may comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin. In certain embodiments, the RAGE polypeptide may comprise a RAGE interdomain linker linked to a RAGE

immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof. For example, certain embodiments of the fusion protein may comprise a first RAGE immunoglobulin domain and a first RAGE interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the second RAGE immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the C_H2 immunoglobulin domain or a portion of a C_H2 domain of an immunoglobulin.

In alternate embodiments of this multi-domain fusion protein, the RAGE polypeptide may comprise the amino acid sequence as set forth in SEQ ID NO: 10, or a sequence at least 90% identical thereto, or the amino acid sequence as set forth in SEQ ID NO: 47, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In other alternate embodiments, the RAGE fusion protein may comprise the amino acid sequence as set forth in at least one of SEQ ID NOs: 32, 33, 34, 35, 36, 37, 56, or 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in certain embodiments, a sequence at least 90% identical to SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57 comprises the polypeptide of SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57 without the C-terminal lysine. Or, other embodiments as described herein may constitute the RAGE fusion protein used in the formulations of the present invention.

A variety of lyoprotectants may be used in the lyophilized RAGE fusion protein formulation. In some embodiments, the lyoprotectant may comprise a non-reducing sugar. For example, the non-reducing sugar may comprise sucrose, mannitol, or trehalose. Also, a variety of buffers may be used in the lyophilized RAGE fusion protein formulation. In certain embodiments, the buffer may comprise histidine.

The lyophilized RAGE fusion protein may comprise additional components. In certain embodiments, the RAGE fusion protein formulation may further comprise at least one of a surfactant, a chelating agent or a bulking agent. In one embodiment, the reconstituted RAGE fusion protein formulation comprises about 40-100 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 2 mM to about 50 mM histidine; about 60 mM to about 65 mM sucrose; about 0.001% to about 0.05% Tween 80; and a pH of about 6.0 to 6.5. For example, the reconstituted RAGE fusion protein formulation may, in certain embodiments, comprise about 40-50 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 10 mM histidine, about 65 mM sucrose, about 0.01% Tween 80, and at a pH of about 6.0. Or, other concentrations of the RAGE fusion protein may be used in the formulations for treatment of RAGE-mediated disorders as is required.

The RAGE fusion protein formulation may comprise a stable therapeutic agent that is formulated for use in a clinic or as a prescription medicine. For example, in certain embodiments, the RAGE fusion protein formulation may exhibit

less than 10%, or less than 5%, or less than 3% decomposition after one week at 40 degrees Centigrade.

Also, the RAGE fusion protein formulation may be stable upon reconstitution in a diluent. In certain embodiments, less than about 10% , or about 5%, or about 4%, or about 3%, or about 2%, or or about 1% of the RAGE fusion protein is present as an aggregate in the RAGE fusion protein formulation.

The reconstituted RAGE fusion protein formulation may be suitable for administration by various routes and as is required for treatment of the RAGE-mediated disorder of interest. Administration of the RAGE fusion protein of the present invention may employ intraperitoneal (IP) injection. Alternatively, the RAGE fusion protein may be administered orally, intranasally, or as an aerosol. In another embodiment, administration is intravenous (IV). The RAGE fusion protein may also be injected subcutaneously. In another embodiment, administration of the RAGE fusion protein is intra-arterial. In another embodiment, administration is sublingual. Also, administration may employ a time-release capsule. In yet another embodiment, administration may be transrectal, as by a suppository or the like. For example, subcutaneous administration may be useful to treat chronic disorders when the self-administration is desirable.

As described in more detail herein, RAGE has been implicated in the pathogenesis of a variety of disease states, and the RAGE fusion proteins of the present invention have been found to be effective in ameliorating such disease states. Thus, the RAGE fusion protein formulations of the present invention may be used to treat a variety of RAGE-mediated disorders.

In certain embodiments, a reconstituted RAGE fusion protein formulation of the present invention may be used to treat a symptom of diabetes or a symptom of diabetic late complications. For example, the symptom of diabetes or diabetic late complications comprises at least one of diabetic nephropathy, diabetic retinopathy, a diabetic foot ulcer, a cardiovascular complication, or diabetic neuropathy.

In other embodiments, a reconstituted RAGE fusion protein formulation of the present invention may be used to treat at least one of amyloidosis, Alzheimer's disease, cancer, kidney failure, or inflammation associated with autoimmunity, inflammatory bowel disease, rheumatoid arthritis, psoriasis, multiple sclerosis, hypoxia, stroke, heart attack, hemorrhagic shock, sepsis, organ transplantation, or impaired wound healing. Or, the reconstituted RAGE fusion protein formulation may be used to treat osteoporosis. For example, in certain embodiments, administration of a RAGE fusion protein formulation of the present invention increases bone density of subject or reduces the rate of a decrease in bone density of a subject.

In some embodiments, the autoimmunity treated using the RAGE fusion protein formulations of the present invention may comprise rejection of at least one of skin cells, pancreatic cells, nerve cells, muscle cells, endothelial cells, heart cells, liver cells, kidney cells, a heart, bone marrow cells, bone, blood cells, artery cells, vein cells, cartilage cells, thyroid, cells, or stem cells. Or, the reconstituted RAGE fusion protein formulation may be used to treat kidney failure.

In certain embodiments, the reconstituted RAGE fusion protein formulation may be used to treat inflammation and/or rejection associated with transplantation of at least one of an organ, a tissue, or a plurality of cells from a first site to a second site. The first and second sites may either be in different subjects, or in the same subject. Transplantation of a variety of different cell types may be improved using the RAGE fusion protein formulations of the present invention.

For example, the transplanted cells, tissue, or organ may comprise a cell, tissue or organ of a pancreas, skin, liver, kidney, heart, bone marrow, blood, bone, muscle, artery, vein, cartilage, thyroid, nervous system, or stem cells.

Examples of using RAGE fusion proteins of the present invention in the treatment of such diseases and disorders are disclosed herein.

For example, a variety of animal models have been used to validate the use of compounds that modulate RAGE as therapeutics. Examples of these models are as follows:

- a) sRAGE inhibited neointimal formation in a rat model of restenosis following arterial injury in both diabetic and normal rats by inhibiting endothelial, smooth muscle and macrophage activation via RAGE (Zhou et al., *Circulation* 107:2238-2243 (2003));
- b) Inhibition of RAGE/ligand interactions, using either sRAGE or an anti-RAGE antibody, reduced amyloid plaque formation in a mouse model of systemic amyloidosis (Yan et al., *Nat. Med.*, 6:643-651 (2000)). Accompanying the reduction in amyloid plaques was a reduction in the inflammatory cytokines, interleukin-6 (IL-6) and macrophage colony stimulating factor (M-CSF) as well as reduced activation of NF- κ B in the treated animals;
- c) RAGE transgenic mice (RAGE overexpressers and RAGE dominant negative expressers) exhibit plaque formation and cognitive deficits in a mouse model of AD (Arancio et al., *EMBO J.*, 23:4096-4105 (2004));
- d) Treatment of diabetic rats with sRAGE reduced vascular permeability (Bonnardel-Phu et al., *Diabetes*, 48:2052-2058 (1999));
- e) Treatment with sRAGE reduced atherosclerotic lesions in diabetic apolipoprotein E-null mice and prevented the functional and morphological indices of diabetic nephropathy in db/db mice (Hudson et al., *Arch. Biochem. Biophys.*, 419:80-88 (2003)); and
- f) sRAGE attenuated the severity of inflammation in a mouse model of collagen-induced arthritis (Hofmann et al., *Genes Immunol.*, 3:123-135 (2002)), a mouse model of experimental allergic encephalomyelitis (Yan et al., *Nat. Med.* 9:28-293 (2003)) and a mouse model of inflammatory bowel disease (Hofmann et al., *Cell*, 97:889-901 (1999)).

Thus, in an embodiment, the RAGE fusion proteins of the present invention may be used to treat a symptom of diabetes and/or complications resulting from diabetes mediated by RAGE. In alternate embodiments, the symptom of diabetes or diabetic late complications may comprise diabetic nephropathy, diabetic retinopathy, a diabetic foot ulcer, a cardiovascular complication of diabetes, or diabetic neuropathy.

Originally identified as a receptor for molecules whose expression is associated with the pathology of diabetes, RAGE itself is essential to the pathophysiology of diabetic complications. In vivo, inhibition of RAGE interaction with its ligand(s) has been shown to be therapeutic in multiple models of diabetic complications and inflammation (Hudson et al., *Arch. Biochem. Biophys.*, 419:80-88 (2003)). For example, a two-month treatment with anti-RAGE antibodies normalized kidney function and reduced abnormal kidney histopathology in diabetic mice (Flyvbjerg et al., *Diabetes* 53:166-172 (2004)). Furthermore, treatment with a soluble form of RAGE (sRAGE) which binds to RAGE ligands and inhibits RAGE/ligand interactions, reduced atherosclerotic lesions in diabetic apolipoprotein E-null mice and attenuated the functional and morphological pathology of diabetic nephropathy in db/db mice (Bucciarelli et al., *Circulation* 106:2827-2835 (2002)).

Also, it has been shown that nonenzymatic glycoxidation of macromolecules ultimately resulting in the formation of advanced glycation endproducts (AGEs) is enhanced at sites of inflammation, in renal failure, in the presence of hyperglycemia and other conditions associated with systemic or local oxidant stress (Dyer et al., *J. Clin. Invest.*, 91:2463-2469 (1993); Reddy et al., *Biochem.*, 34:10872-10878 (1995); Dyer et al., *J. Biol. Chem.*, 266:11654-11660 (1991); Degenhardt et al., *Cell Mol. Biol.*, 44:1139-1145 (1998)). Accumulation of AGEs in the vasculature can occur focally, as in the joint amyloid composed of AGE- β_2 -microglobulin found in patients with dialysis-related amyloidosis (Miyata et al., *J. Clin. Invest.*, 92:1243-1252 (1993); Miyata et al., *J. Clin. Invest.*, 98:1088-1094 (1996)), or generally, as exemplified by the vasculature and tissues of patients with diabetes (Schmidt et al., *Nature Med.*, 1:1002-1004 (1995)). The progressive accumulation of AGEs over time in patients with diabetes suggests that endogenous clearance mechanisms are not able to function effectively at sites of AGE deposition. Such accumulated AGEs have the capacity to alter cellular properties by a number of mechanisms. Although RAGE is expressed at low levels in normal tissues and vasculature, in an environment where the receptor's ligands accumulate, it has been shown that RAGE becomes upregulated (Li et al., *J. Biol. Chem.*, 272:16498-16506 (1997); Li et al., *J. Biol. Chem.*, 273:30870-30878 (1998); Tanaka et al., *J. Biol. Chem.*, 275:25781-25790 (2000)). RAGE expression is increased in endothelium, smooth muscle cells and infiltrating mononuclear phagocytes in diabetic vasculature. Also, studies in cell culture have demonstrated that AGE-RAGE interaction causes changes in cellular properties important in vascular homeostasis.

Use of the RAGE fusion proteins in the treatment of diabetes related pathology is illustrated in FIG. 14. The RAGE fusion protein TTP-4000 was evaluated in a diabetic rat model of restenosis which involved measuring smooth muscle proliferation and intimal expansion following vascular injury. As illustrated in FIG. 14, TTP-4000 treatment may significantly reduce the intima/media (I/M) ratio (FIG. 14A; Table 1) in diabetes-associated restenosis in a dose-responsive manner. Also, TTP-4000 treatment may significantly reduce restenosis-associated vascular smooth muscle cell proliferation in a dose-responsive manner (FIG. 14B).

TABLE 1

Effect of TTP-4000 in Rat Model of Restenosis			
	IgG (n = 9)	TTP-4000 (n = 9) Low dose** (0.3 mg/animal qod x 4)	TTP-4000 (n = 9) High dose** (1.0 mg/animal qod x 4)
Intimal area (mm ²)	0.2 ± 0.03	0.18 ± 0.04	0.16 ± 0.02
Medial area (mm ²)	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
I/M ratio	1.71 ± 0.27	1.61 ± 0.26	1.44* ± 0.15

*P < 0.05;

**For both high and low dose, a loading dose of 3 mg/animal was used.

In other embodiments, the RAGE fusion proteins of the present invention may also be used to treat or reverse amyloidoses and Alzheimer's disease. RAGE is a receptor for amyloid beta (A β) as well as other amyloidogenic proteins including SAA and amylin (Yan et al., *Nature*, 382:685-691 (1996); Yan et al., *Proc. Natl. Acad. Sci., USA*, 94:5296-5301 (1997); Yan et al., *Nat. Med.*, 6:643-651 (2000); Sousa et al., *Lab Invest.*, 80:1101-1110 (2000)). Also, the RAGE ligands, including AGEs, S100b and A β proteins, are found in tissue surrounding the senile plaque in man (Luth et al., *Cereb.*

Cortex 15:211-220 (2005); Petzold et al., *Neurosci. Lett.*, 336:167-170 (2003); Sasaki et al., *Brain Res.*, 12:256-262 (2001); Yan et al., *Restor. Neurol. Neurosci.*, 12:167-173 (1998). It has been shown that RAGE binds β -sheet fibrillar material regardless of the composition of the subunits (amyloid- β peptide, amylin, serum amyloid A, prion-derived peptide) (Yan et al., *Nature*, 382:685-691 (1996); Yan et al., *Nat. Med.*, 6:643-651 (2000)). In addition, deposition of amyloid has been shown to result in enhanced expression of RAGE. For example, in the brains of patients with Alzheimer's disease (AD), RAGE expression increases in neurons and glia (Yan, et al., *Nature* 382:685-691 (1996)). Concurrent with expression of RAGE ligands, RAGE is upregulated in astrocytes and microglial cells in the hippocampus of individuals with AD but is not upregulated in individuals that do not have AD (Lue et al., *Exp. Neurol.*, 171:29-45 (2001)). These findings suggest that cells expressing RAGE are activated via RAGE/RAGE ligand interactions in the vicinity of the senile plaque. Also, in vitro, A β -mediated activation of microglial cells can be blocked with antibodies directed against the ligand-binding domain of RAGE (Yan et al., *Proc. Natl. Acad. Sci., USA*, 94:5296-5301 (1997)). It has also been demonstrated that RAGE can serve as a focal point for fibril assembly (Deane et al., *Nat. Med.* 9:907-913 (2003)).

Also, in vivo inhibition of RAGE/ligand interactions using either sRAGE or an anti-RAGE antibody can reduce amyloid plaque formation in a mouse model of systemic amyloidosis (Yan et al., *Nat. Med.*, 6:643-651 (2000)). Double transgenic mice that over-express human RAGE and human amyloid precursor protein (APP) with the Swedish and London mutations (mutant hAPP) in neurons develop learning defects and neuropathological abnormalities earlier than their single mutant hAPP transgenic counterparts. In contrast, double transgenic mice with diminished A β signaling capacity due to neurons expressing a dominant negative form of RAGE on the same mutant hAPP background, show a delayed onset of neuropathological and learning abnormalities compared to their single APP transgenic counterpart (Arancio et al., *EMBO J.*, 23:4096-4105 (2004)).

In addition, inhibition of RAGE-amyloid interaction has been shown to decrease expression of cellular RAGE and cell stress markers (as well as NF- κ B activation), and diminish amyloid deposition (Yan et al., *Nat. Med.*, 6:643-651 (2000)) suggesting a role for RAGE-amyloid interaction in both perturbation of cellular properties in an environment enriched for amyloid (even at early stages) as well as in amyloid accumulation.

Thus, the RAGE fusion proteins of the present invention may also be used to treat reduce amyloidosis and to reduce amyloid plaques and cognitive dysfunction associated with Alzheimer's Disease (AD). As described above, sRAGE has been shown to reduce both amyloid plaque formation in the brain and subsequent increase in inflammatory markers in an animal model of AD. FIGS. 15A and 15B show that mice that have AD, and are treated for 3 months with either TTP-4000 or mouse sRAGE had fewer amyloid beta (A β) plaques and less cognitive dysfunction than animals that received a vehicle or a human IgG negative control (IgG1). Like sRAGE, TTP-4000 may also reduce the inflammatory cytokines IL-1 and TNF- α (data not shown) associated with AD.

Also, RAGE fusion proteins of the present invention may be used to treat atherosclerosis and other cardiovascular disorders. Thus, it has been shown that ischemic heart disease is particularly high in patients with diabetes (Robertson, et al., *Lab Invest.*, 18:538-551 (1968); Kannel et al., *J. Am. Med. Assoc.*, 241:2035-2038 (1979); Kannel et al., *Diab. Care*, 2:120-126 (1979)). In addition, studies have shown that ath-

erosclerosis in patients with diabetes is more accelerated and extensive than in patients not suffering from diabetes (see e.g. Waller et al., *Am. J. Med.*, 69:498-506 (1980); Crall et al., *Am. J. Med.* 64:221-230 (1978); Hamby et al., *Chest*, 2:251-257 (1976); and Pyorala et al., *Diab. Metab. Rev.*, 3:463-524 (1978)). Although the reasons for accelerated atherosclerosis in the setting of diabetes are many, it has been shown that reduction of AGEs can reduce plaque formation.

For example, the RAGE fusion proteins of the present invention may also be used to treat stroke. When TTP-4000 was compared to sRAGE in a disease relevant animal model of stroke, TTP-4000 was found to provide a significantly greater reduction in infarct volume. In this model, the middle carotid artery of a mouse is ligated and then reperfused to form an infarct. To assess the efficacy of RAGE fusion proteins to treat or prevent stroke, mice were treated with sRAGE or TTP-4000 or control immunoglobulin just prior to reperfusion. As can be seen in Table 2, TTP-4000 was more efficacious than sRAGE in limiting the area of infarct in these animals suggesting that TTP-4000, because of its better half-life in plasma, was able to maintain greater protection than sRAGE.

TABLE 2

Reduction of Infarct in Stroke	
	% Reduction of Infarct**
sRAGE	15%*
TTP-4000 (300 μ g)	38%*
TTP-4000 (300 μ g)	21%*
TTP-4000 (300 μ g)	10%*
IgG Isotype control (300 μ g)	4%

*Significant to $p < 0.001$;

**Compared to saline

In another embodiment, the RAGE fusion proteins of the present invention may be used to treat cancer. In one embodiment, the cancer treated using the RAGE fusion proteins of the present invention comprises cancer cells that express RAGE. For example, cancers that may be treated with the RAGE fusion protein of the present invention include some lung cancers, some gliomas, some papillomas, and the like. Amphotericin is a high mobility group I nonhistone chromosomal DNA binding protein (Rauvala et al., *J. Biol. Chem.*, 262:16625-16635 (1987); Parkikinen et al., *J. Biol. Chem.* 268:19726-19738 (1993)) which has been shown to interact with RAGE. It has been shown that amphotericin promotes neurite outgrowth, as well as serving as a surface for assembly of protease complexes in the fibrinolytic system (also known to contribute to cell mobility). In addition, a local tumor growth inhibitory effect of blocking RAGE has been observed in a primary tumor model (C6 glioma), the Lewis lung metastasis model (Taguchi et al., *Nature* 405:354-360 (2000)), and spontaneously arising papillomas in mice expressing the v-Ha-ras transgene (Leder et al., *Proc. Natl. Acad. Sci.*, 87:9178-9182 (1990)).

In yet another embodiment, the RAGE fusion proteins of the present invention may be used to treat inflammation. In alternate embodiments, the RAGE fusion proteins of the present invention may be used to treat inflammation associated with inflammatory bowel disease, inflammation associated with rheumatoid arthritis, inflammation associated with psoriasis, inflammation associated with multiple sclerosis, inflammation associated with hypoxia, inflammation associated with stroke, inflammation associated with heart attack, inflammation associated with hemorrhagic shock, inflamma-

tion associated with sepsis, inflammation associated with organ transplantation, inflammation associated with impaired wound healing, or inflammation associated with rejection of self (e.g., autoimmune) or non-self (e.g., transplanted) cells, tissue, or organs.

For example, following thrombolytic treatment, inflammatory cells such as granulocytes infiltrate the ischemic tissue and produce oxygen radicals that can destroy more cells than were killed by the hypoxia. Inhibiting the receptor on the neutrophil responsible for the neutrophils being able to infiltrate the tissue with antibodies or other protein antagonists has been shown to ameliorate the response. Since RAGE is a ligand for this neutrophil receptor, a RAGE fusion protein containing a fragment of RAGE may act as a decoy and prevent the neutrophil from trafficking to the reperfused site and thus prevent further tissue destruction. The role of RAGE in prevention of inflammation may be indicated by studies showing that sRAGE inhibited neointimal expansion in a rat model of restenosis following arterial injury in both diabetic and normal rats, presumably by inhibiting endothelial, smooth muscle cell proliferation and macrophage activation via RAGE (Zhou et al., *Circulation*, 107:2238-2243 (2003)). In addition, sRAGE inhibited models of inflammation including delayed-type hypersensitivity, experimental autoimmune encephalitis and inflammatory bowel disease (Hofman et al., *Cell*, 97:889-901 (1999)).

In an embodiment, the RAGE fusion proteins of the present invention may be used to treat auto-immune based disorders. For example, in an embodiment, the RAGE fusion proteins of the present invention may be used to treat kidney failure. Thus, the RAGE fusion proteins of the present invention may be used to treat systemic lupus nephritis or inflammatory lupus nephritis. For example, the S100/calgranulins have been shown to comprise a family of closely related calcium-binding polypeptides characterized by two EF-hand regions linked by a connecting peptide (Schafer et al., *TIBS*, 21:134-140 (1996); Zimmer et al., *Brain Res. Bull.*, 37:417-429 (1995); Rammes et al., *J. Biol. Chem.*, 272:9496-9502 (1997); Luger et al., *Eur. J. Clin. Invest.*, 25:659-664 (1995)). Although they lack signal peptides, it has long been known that S100/calgranulins gain access to the extracellular space, especially at sites of chronic immune/inflammatory responses, as in cystic fibrosis and rheumatoid arthritis. RAGE is a receptor for many members of the S100/calgranulin family, mediating their proinflammatory effects on cells such as lymphocytes and mononuclear phagocytes. Also, studies on delayed-type hypersensitivity response, colitis in IL-10 null mice, collagen-induced arthritis, and experimental autoimmune encephalitis models suggest that RAGE-ligand interaction (presumably with S-100/calgranulins) has a proximal role in the inflammatory cascade.

Type I diabetes is an autoimmune disorder that may be prevented or ameliorated by treatment with the RAGE fusion proteins of the present invention. For example, it has been shown that sRAGE may allow for the transfer of splenocytes from non-obese diabetic (NOD) mice to NOD-mice with severe combined immunodeficiency (NOD-scid mice). NOD-scid mice do not display diabetes spontaneously, but require the presence of immunocytes capable of destroying islet cells such that diabetes is then induced. It was found that NOD-scid recipients treated with sRAGE displayed reduced onset of diabetes induced by splenocytes transferred from a diabetic (NOD) mouse as compared to NOD-scid recipients not treated with sRAGE (U.S. Patent Publication 2002/0122799). As stated in US 2002/0122799, the experimental results using sRAGE in this model are relevant to human

disease such as clinical settings in which future immune therapies and islet transplantation may occur.

Thus, in an embodiment, a RAGE fusion protein of the present invention may be used to treat inflammation associated with transplantation of at least one of an organ, a tissue, or a plurality of cells from a first site to a second site. The first and second sites may be in different subjects, or in the same subject. In alternate embodiments, the transplanted cells, tissue or organ comprise cells of a pancreas, skin, liver, kidney, heart, lung, bone marrow, blood, bone, muscle, endothelial cells, artery, vein, cartilage, thyroid, nervous system, or stem cells. For example, administration of the RAGE fusion proteins of the present invention may be used to facilitate transplantation of islet cells from a first non-diabetic subject to a second diabetic subject.

In another embodiment, the present invention may provide a method of treating osteoporosis by administering to a subject a therapeutically effective amount of a RAGE fusion protein of the present invention. (Zhou et al., *J. Exp. Med.*, 203:1067 - 1080 (2006)). In an embodiment, the method of treating osteoporosis may further comprise the step of increasing bone density of the subject or reducing the rate of decrease in bone density of a subject.

Thus, in various selected embodiments, the present invention may provide a method for inhibiting the interaction of an AGE with RAGE in a subject by administering to the subject a therapeutically effective amount of a RAGE fusion protein of the present invention. The subject treated using the RAGE fusion proteins of the present invention may be an animal. In an embodiment, the subject is a human. The subject may be suffering from an AGE-related disease such as diabetes, diabetic complications such as nephropathy, neuropathy, retinopathy, foot ulcer, amyloidoses, or renal failure, and inflammation. Or, the subject may be an individual with Alzheimer's disease. In an alternative embodiment, the subject may be an individual with cancer. In yet other embodiments, the subject may be suffering from systemic lupus erythematosus or inflammatory lupus nephritis. Other diseases may be mediated by RAGE and thus, may be treated using the RAGE fusion proteins of the present invention. Thus, in additional alternative embodiments of the present invention, the RAGE fusion proteins may be used for treatment of Crohn's disease, arthritis, vasculitis, nephropathies, retinopathies, and neuropathies in human or animal subjects. In other embodiments, inflammation involving both autoimmune responses (e.g., rejection of self) and non-autoimmune responses (e.g., rejection of non-self) may be mediated by RAGE and thus, may be treated using the RAGE fusion proteins of the present invention.

A therapeutically effective amount may comprise an amount which is capable of preventing the interaction of RAGE with an AGE or other types of endogenous RAGE ligands in a subject. Accordingly, the amount will vary with the subject being treated. Administration of the compound may be hourly, daily, weekly, monthly, yearly, or as a single event. In various alternative embodiments, the effective amount of the RAGE fusion protein may range from about 1 ng/kg body weight to about 100 mg/kg body weight, or from about 10 μ g/kg body weight to about 50 mg/kg body weight, or from about 100 μ g/kg body weight to about 20 mg/kg body weight. The actual effective amount may be established by dose/response assays using methods standard in the art (Johnson et al., *Diabetes*. 42: 1179, (1993)). Thus, as is known to those in the art, the effective amount may depend on bioavailability, bioactivity, and biodegradability of the compound.

Compositions

The present invention may comprise a composition comprising a RAGE fusion protein of the present invention mixed with a pharmaceutically acceptable carrier. The RAGE fusion protein may comprise a RAGE polypeptide linked to a second, non-RAGE polypeptide. In one embodiment, the RAGE fusion protein may comprise a RAGE ligand binding site. In an embodiment, the ligand binding site comprises the most N-terminal domain of the RAGE fusion protein. The RAGE ligand binding site may comprise the V domain of RAGE, or a portion thereof. In an embodiment, the RAGE ligand binding site comprises SEQ ID NO: 9 or a sequence at least 90% identical thereto, or SEQ ID NO: 10 or a sequence at least 90% identical thereto, or SEQ ID NO: 47 or a sequence at least 90% identical thereto.

In an embodiment, the RAGE polypeptide may be linked to a polypeptide comprising an immunoglobulin domain or a portion (e.g., a fragment thereof) of an immunoglobulin domain. In one embodiment, the polypeptide comprising an immunoglobulin domain comprises at least a portion of at least one of the C_H2 or the C_H3 domains of a human IgG.

In certain embodiments, the RAGE fusion protein comprises an amino acid sequence as set forth in SEQ ID NO: 56 or SEQ ID NO: 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in some embodiments, a sequence at least 90% identical to SEQ ID NO: 56 or SEQ ID NO: 57 comprises the sequence of SEQ ID NO: 56 or SEQ ID NO: 57 without the C-terminal lysine.

The RAGE protein or polypeptide may comprise full-length human RAGE (e.g., SEQ ID NO: 1), or a fragment of human RAGE. In an embodiment, the RAGE polypeptide does not include any signal sequence residues. The signal sequence of RAGE may comprise either residues 1-22 or residues 1-23 of full length RAGE (SEQ ID NO: 1). In alternate embodiments, the RAGE polypeptide may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to human RAGE, or a fragment thereof. For example, in one embodiment, the RAGE polypeptide may comprise human RAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neepet et al., (1992)). Or, the human RAGE may comprise full-length RAGE with the signal sequence removed (e.g., SEQ ID NO: 2 or SEQ ID NO: 3) (FIGS. 1A and 1B) or a portion of that amino acid sequence.

The RAGE fusion proteins of the present invention may also comprise sRAGE (e.g., SEQ ID NO: 4), a polypeptide at least 90% identical to sRAGE, or a fragment of sRAGE. For example, the RAGE polypeptide may comprise human sRAGE, or a fragment thereof, with Glycine as the first residue rather than a Methionine (see e.g., Neepet et al., (1992)). Or, the human RAGE may comprise sRAGE with the signal sequence removed (See e.g., SEQ ID NO: 5, SEQ ID NO: 6 or SEQ ID NO: 45 in FIG. 1) or a portion of that amino acid sequence. In other embodiments, the RAGE protein may comprise a V domain (See e.g., SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 46 in FIG. 1). Or, a sequence at least 90% identical to the V domain or a fragment thereof may be used. Or, the RAGE protein may comprise a fragment of RAGE comprising a portion of the V domain (See e.g., SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 47 in FIG. 1). In an embodiment, the ligand binding site may comprise SEQ ID NO: 9, or a sequence at least 90% identical thereto, or SEQ ID NO: 10, or a sequence at least 90% identical thereto, or SEQ ID NO: 47, or a sequence at least 90% identical thereto. In yet another embodiment, the RAGE fragment is a synthetic peptide.

For example, the RAGE polypeptide may comprise amino acids 23-116 of human RAGE (SEQ ID NO: 7) or a sequence at least 90% identical thereto, or amino acids 24-116 of human RAGE (SEQ ID NO: 8) or a sequence at least 90% identical thereto, or amino acids 24-116 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 46), or a sequence at least 90% identical thereto, corresponding to the V domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 124-221 of human RAGE (SEQ ID NO: 11) or a sequence at least 90% identical thereto, corresponding to the C1 domain of RAGE. In another embodiment, the RAGE polypeptide may comprise amino acids 227-317 of human RAGE (SEQ ID NO: 12) or a sequence at least 90% identical thereto, corresponding to the C2 domain of RAGE. Or, the RAGE polypeptide may comprise amino acids 23-123 of human RAGE (SEQ ID NO: 13) or a sequence at least 90% identical thereto, or amino acids 24-123 of human RAGE (SEQ ID NO: 14) or a sequence at least 90% identical thereto, corresponding to the V domain of RAGE and a downstream interdomain linker. Or, the RAGE polypeptide may comprise amino acids 24-123 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 48), or a sequence at least 90% identical thereto. Or, the RAGE polypeptide may comprise amino acids 23-226 of human RAGE (SEQ ID NO: 17) or a sequence at least 90% identical thereto, or amino acids 24-226 of human RAGE (SEQ ID NO: 18) or a sequence at least 90% identical thereto, corresponding to the V-domain, the C1 domain and the interdomain linker linking these two domains. Or, the RAGE polypeptide may comprise amino acids 24-226 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 50), or a sequence 90% identical thereto. Or, the RAGE polypeptide may comprise amino acids 23-339 of human RAGE (SEQ ID NO: 5) or a sequence at least 90% identical thereto, or 24-339 of human RAGE (SEQ ID NO: 6) or a sequence at least 90% identical thereto, corresponding to sRAGE (i.e., encoding the V, C1, and C2 domains and interdomain linkers). Or, the RAGE polypeptide may comprise amino acids 24-339 of human RAGE where Q24 cyclizes to form pE (SEQ ID NO: 45), or a sequence at least 90% identical thereto. Or, fragments of each of these sequences may be used.

In another embodiment, the ligand binding site may comprise amino acids 22-51 of SEQ ID NO. 1. In another embodiment, the ligand binding site may comprise amino acids 23-51 of SEQ. ID NO: 1. In another embodiment, the ligand binding site may comprise amino acids 31-51 of SEQ ID NO: 1. In another embodiment, the ligand binding site may comprise amino acids 31-116 of SEQ ID NO: 1. For example, the ligand binding site may comprise, a RAGE V domain or a portion thereof such as the RAGE ligand binding domain (e.g., amino acids 1-118, 23-118, 24-118, 31-118, 1-116, 23-116, 24-116, 31-116, 1-54, 23-54, 24-54, 31-54, 1-53, 23-53, 24-53, or 31-53 of SEQ ID NO: 1, or fragments thereof) (FIG. 1). Or fragments of the polypeptides that functionally bind a RAGE ligand may be used. Or, a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the RAGE V domain or a fragment thereof (e.g., as described above) may be used. Further, as is known in the art, in embodiments where the N-terminus of the fusion protein is glutamine, as for example upon removal of the signal sequence comprising residues 1-23 of SEQ ID NO: 1 (e.g., Q24 for a polypeptide comprise amino acids 24-118 or SEQ ID NO: 1), the glutamine may cyclize to form pyroglutamic acid (pE).

The RAGE fusion protein may include several types of peptides that are not derived from RAGE or a fragment thereof. The second polypeptide of the RAGE fusion protein

may comprise a polypeptide derived from an immunoglobulin. The heavy chain (or portion thereof) may be derived from any one of the known heavy chain isotypes: IgG (γ), IgM (μ), IgD (δ), IgE (ϵ) or IgA (α). In addition, the heavy chain (or portion thereof) may be derived from any one of the known heavy chain subtypes: IgG1 (γ 1), IgG2 (γ 2), IgG3 (γ 3), IgG4 (γ 4), IgA1 (α 1), IgA2 (α 2), or mutations of these isotypes or subtypes that alter the biological activity. The second polypeptide may comprise the C_{H2} and C_{H3} domains of a human IgG1 or a portion of either, or both, of these domains. As an example embodiments, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 40 or a portion thereof. In an embodiment, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38, or a portion thereof. For example, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40 with the terminal lysine (K) removed. The immunoglobulin peptide may be encoded by the nucleic acid sequence of SEQ ID NO: 39 or SEQ ID NO: 41. The immunoglobulin sequence in SEQ ID NO: 38 or SEQ ID NO: 40 may also be encoded by SEQ ID NO: 52 or SEQ ID NO: 53, respectively.

The Fc portion of the immunoglobulin chain may be proinflammatory *in vivo*. Thus, in one embodiment, the RAGE fusion protein of the present invention comprises an interdomain linker derived from RAGE rather than an interdomain hinge polypeptide derived from an immunoglobulin.

Thus in one embodiment, the RAGE fusion protein may further comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_{H2} domain of an immunoglobulin, or a fragment thereof. In one embodiment, the C_{H2} domain, or a fragment thereof comprises SEQ ID NO: 42. In an embodiment, the fragment of SEQ ID NO: 42 comprises SEQ ID NO: 42 with the first ten amino acids removed.

In one embodiment, the RAGE polypeptide comprises a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_{H2} domain of an immunoglobulin, or a fragment thereof. The polypeptide comprising a C_{H2} domain of an immunoglobulin, or a portion thereof, may comprise the C_{H2} and C_{H3} domains of a human IgG1, or a portion of both, or either, of these domains. As example embodiments, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1, or a portion thereof, may comprise SEQ ID NO: 40 or a portion thereof. In an embodiment, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38, or a portion thereof. For example, the polypeptide comprising the C_{H2} and C_{H3} domains of a human IgG1 or a portion thereof may comprise SEQ ID NO: 38 or SEQ ID NO: 40 with the terminal lysine (K) removed. The RAGE fusion protein of the present invention may comprise a single or multiple domains from RAGE. Also, the RAGE polypeptide comprising an interdomain linker linked to a RAGE immunoglobulin domain may comprise a fragment of a full-length RAGE protein. For example, in one embodiment, the RAGE fusion protein may comprise two immunoglobulin domains derived from RAGE protein and two immunoglobulin domains derived from a human Fc polypeptide. The RAGE fusion protein may comprise a first RAGE immunoglobulin domain and a first interdomain linker linked to a second RAGE immunoglobulin domain and a

second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the RAGE second immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the polypeptide comprising a C_{H2} immunoglobulin domain or fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-251 of human RAGE (SEQ ID NO: 19) or a sequence at least 90% identical thereto, or amino acids 24-251 of human RAGE (SEQ ID NO: 20) or a sequence at least 90% identical thereto, or amino acids 24-251 of human RAGE where Q24 cyclizes to form pE, or a sequence at least 90% identical thereto (SEQ ID NO: 51), corresponding to the V-domain, the C1 domain, the interdomain linker linking these two domains, and a second interdomain linker downstream of C1. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 30 or a fragment thereof may encode for a four domain RAGE fusion protein. In another embodiment, nucleic acid construct comprising SEQ ID NO: 54 may encode for a four domain RAGE fusion protein, where silent base changes for the codons that encode for proline (CCG to CCC) and glycine (GGT to GGG) at the C-terminus of the sequence are entered to remove a cryptic RNA splice site near the terminal codon (i.e., at nucleotides 1375-1380 of SEQ ID NO: 30 are modified to generate SEQ ID NO: 54).

Alternatively, a three domain RAGE fusion protein may comprise one immunoglobulin domain derived from RAGE and two immunoglobulin domains derived from a human Fc polypeptide. For example, the RAGE fusion protein may comprise a single RAGE immunoglobulin domain linked via a RAGE interdomain linker to the N-terminal amino acid of the polypeptide comprising a C_{H2} immunoglobulin domain or a fragment thereof. For example, the RAGE polypeptide may comprise amino acids 23-136 of human RAGE (SEQ ID NO: 15) or a sequence at least 90% identical thereto or amino acids 24-136 of human RAGE (SEQ ID NO: 16) or a sequence at least 90% identical thereto, or amino acids 24-136 of human RAGE where Q24 cyclizes to form pE, or a sequence at least 90% identical thereto (SEQ ID NO: 49), corresponding to the V domain of RAGE and a downstream interdomain linker. In one embodiment, a nucleic acid construct comprising SEQ ID NO: 31 or a fragment thereof may encode for a three domain RAGE fusion protein. In another embodiment, nucleic acid construct comprising SEQ ID NO: 55 may encode for a three domain RAGE fusion protein, where silent base changes for the codons that encode for proline (CCG to CCC) and glycine (GGT to GGG) at the C-terminus of the sequence are entered to remove a cryptic RNA splice site near the terminal codon (i.e., nucleotides 1030-1035 of SEQ ID NO: 31 are modified to generate SEQ ID NO: 55).

A RAGE interdomain linker fragment may comprise a peptide sequence that is naturally downstream of, and thus, linked to, a RAGE immunoglobulin domain. For example, for the RAGE V domain, the interdomain linker may comprise amino acid sequences that are naturally downstream from the V domain. In an embodiment, the linker may comprise SEQ ID NO: 21, corresponding to amino acids 117-123 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, an interdomain linker comprising several amino

acids (e.g., 1-3, 1-5, or 1-10, or 1-15 amino acids) upstream and downstream of SEQ ID NO: 21 may be used. Thus, in one embodiment, the interdomain linker comprises SEQ ID NO: 23 comprising amino acids 117-136 of full-length RAGE. Or, fragments of SEQ ID NO: 21 deleting, for example, 1, 2, or 3, amino acids from either end of the linker may be used. In alternate embodiments, the linker may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 21 or SEQ ID NO: 23.

For the RAGE C1 domain, the linker may comprise a peptide sequence that is naturally downstream of the C1 domain. In an embodiment, the linker may comprise SEQ ID NO: 22, corresponding to amino acids 222-251 of full-length RAGE. Or, the linker may comprise a peptide having additional portions of the natural RAGE sequence. For example, a linker comprising several (1-3, 1-5, or 1-10, or 1-15 amino acids) amino acids upstream and downstream of SEQ ID NO: 22 may be used. Or, fragments of SEQ ID NO: 22 may be used, deleting for example, 1-3, 1-5, or 1-10, or 1-15 amino acids from either end of the linker. For example, in one embodiment, a RAGE interdomain linker may comprise SEQ ID NO: 24, corresponding to amino acids 222-226. In alternate embodiments, the linker may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 22 or SEQ ID NO: 24.

Or an interdomain linker may comprise SEQ ID NO: 44, corresponding to RAGE amino acids 318-342. In alternate embodiments, the linker may comprise a sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 44.

Pharmaceutically acceptable carriers may comprise any of the standard pharmaceutically accepted carriers known in the art. In one embodiment, the pharmaceutical carrier may be a liquid and the RAGE fusion protein or nucleic acid construct may be in the form of a solution. In another embodiment, the pharmaceutically acceptable carrier may be a solid in the form of a powder, a lyophilized powder, or a tablet. Or, the pharmaceutical carrier may be a gel, suppository, or cream. In alternate embodiments, the carrier may comprise a liposome, a microcapsule, a polymer encapsulated cell, or a virus. Thus, the term pharmaceutically acceptable carrier encompasses, but is not limited to, any of the standard pharmaceutically accepted carriers, such as water, alcohols, phosphate buffered saline solution, sugars (e.g., sucrose or mannitol), oils or emulsions such as oil/water emulsions or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules.

In certain embodiments, the RAGE fusion proteins may be present in a neutral form (including zwitter ionic forms) or as a positively or negatively-charged species. In some embodiments, the RAGE fusion proteins may be complexed with a counterion to form a pharmaceutically acceptable salt.

The terms "pharmaceutically acceptable salt" refer to a complex comprising one or more RAGE fusion proteins and one or more counterions, where the counterions are derived from pharmaceutically acceptable inorganic and organic acids and bases.

Pharmaceutically acceptable inorganic bases include metallic ions. More preferred metallic ions include, but are not limited to, appropriate alkali metal salts, alkaline earth metal salts and other physiological acceptable metal ions. Salts derived from inorganic bases include aluminum, ammonium, calcium, cobalt, nickel, molybdenum, vanadium, manganese, chromium, selenium, tin, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, rubidium, sodium, and zinc, and in their usual valences.

Pharmaceutically acceptable acid addition salts of the RAGE fusion proteins of the present invention can be prepared from the following acids, including, without limitation formic, acetic, acetamidobenzoic, adipic, ascorbic, boric, propionic, benzoic, camphoric, carbonic, cyclamic, dehydrocholic, malonic, edetic, ethylsulfuric, fendizoic, metaphosphoric, succinic, glycolic, gluconic, lactic, malic, tartaric, tannic, citric, nitric, ascorbic, glucuronic, maleic, folic, fumaric, propionic, pyruvic, aspartic, glutamic, benzoic, hydrochloric, hydrobromic, hydroiodic, lysine, isocitric, trifluoroacetic, pamoic, propionic, anthranilic, mesylic, orotic, oxalic, oxalacetic, oleic, stearic, salicylic, aminosalicilic, silicate, p-hydroxybenzoic, nicotinic, phenylacetic, mandelic, embonic, sulfonic, methanesulfonic, phosphoric, phosphonic, ethanesulfonic, ethanedisulfonic, ammonium, benzenesulfonic, pantothenic, naphthalenesulfonic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, sulfuric, nitric, nitrous, sulfuric acid monomethyl ester, cyclohexylaminosulfonic, β -hydroxybutyric, glycine, glycyglycine, glutamic, cacodylate, diaminohexanoic, camphorsulfonic, gluconic, thiocyanic, oxoglutaric, pyridoxal 5-phosphate, chlorophenoxyacetic, undecanoic, N-acetyl-L-aspartic, galactaric and galacturonic acids.

Pharmaceutically acceptable organic bases include trimethylamine, diethylamine, N,N'-dibenzylethylenediamine, chlorprocaine, choline, dibenzylamine, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), procaine, cyclic amines, quaternary ammonium cations, arginine, betaine, caffeine, clemizole, 2-ethylaminoethanol, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanedi- amine, butylamine, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, ethylglucamine, glucamine, glucosamine, histidine, hydrabamine, imidazole, isopropylamine, methylglucamine, morpholine, piperazine, pyridine, pyridoxine, neodymium, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, tripropylamine, triethanolamine, tromethamine, methylamine, taurine, cholate, 6-amino-2-methyl-2-heptanol, 2-amino-2-methyl-1,3-propanediol, 2-amino-2-methyl-1-propanol, aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, aromatic acids, aliphatic and aromatic sulfonic acids, strontium, tricine, hydrazine, phenylcyclohexylamine, 2-(N-morpholino)ethanesulfonic acid, bis(2-hydroxyethyl)amino-tris (hydroxymethyl)methane, N-(2-acetamido)-2-aminoethanesulfonic acid, 1,4-piperazinediethanesulfonic acid, 3-morpholino-2-hydroxypropanesulfonic acid, 1,3-bis [tris(hydroxymethyl)methylamino]propane, 4-morpholinepropanesulfonic acid, 4-(2-hydroxyethylpiperazine-1-ethanesulfonic acid, 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid, N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, 4-(N-morpholino) butanesulfonic acid, 3-(N,N-bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid, 2-hydroxy-3-[tris (hydroxymethyl)methylamino]-1-propanesulfonic acid, 4-(2-hydroxyethylpiperazine-1-(2-hydroxypropanesulfonic acid), piperazine-1,4-bis(2-hydroxypropanesulfonic acid) dihydrate, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid, N,N-bis(2-hydroxyethyl)glycine, N-(2-hydroxyethyl) piperazine-N'-(4-butanedisulfonic acid), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid, N-tris(Hydroxymethyl)methyl-4-aminobutanedisulfonic acid, N-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid, 2-(cyclohexylamino) ethanesulfonic acid, 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid, 3-(cyclohexylamino)-1-propanesulfonic acid, N-(2-acetamido)iminodiacetic acid,

4-(cyclohexylamino)-1-butanefulfonic acid, N-[tris(hydroxymethyl)methyl]glycine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, and trometamol.

Administration of the RAGE fusion proteins of the present invention may employ various routes. Thus, administration of the RAGE fusion protein of the present invention may employ intraperitoneal (IP) injection. Alternatively, the RAGE fusion protein may be administered orally, intranasally, or as an aerosol. In another embodiment, administration is intravenous (IV). The RAGE fusion protein may also be injected subcutaneously. In another embodiment, administration of the RAGE fusion protein is intra-arterial. In another embodiment, administration is sublingual. Also, administration may employ a time-release capsule. For example, subcutaneous administration may be useful to treat chronic disorders when the self-administration is desirable.

In a further aspect of the present invention, the RAGE fusion proteins of the invention may be utilized in adjuvant therapeutic or combination therapeutic treatments with other known therapeutic agents. The following is a non-exhaustive listing of adjuvants and additional therapeutic agents which may be utilized in combination with the RAGE fusion protein modulators of the present invention:

Pharmacologic Classifications of Anticancer Agents:

1. Alkylating agents: Cyclophosphamide, nitrosoureas, carboplatin, cisplatin, procarbazine
2. Antibiotics: Bleomycin, Daunorubicin, Doxorubicin
3. Antimetabolites: Methotrexate, Cytarabine, Fluorouracil, Azathioprine, 6-Mercaptopurine, and cytotoxic cancer chemotherapeutic agents
4. Plant alkaloids: Vinblastine, Vincristine, Etoposide, Paclitaxel,
5. Hormones: Tamoxifen, Octreotide acetate, Finasteride, Flutamide
6. Biologic response modifiers: Interferons, Interleukins

Pharmacologic Classifications of Treatment for Rheumatoid Arthritis

1. Analgesics: Aspirin
2. NSAIDs (Nonsteroidal anti-inflammatory drugs): Ibuprofen, Naproxen, Diclofenac
3. DMARDs (Disease-Modifying Antirheumatic drugs): Methotrexate, gold preparations, hydroxychloroquine, sulfasalazine
4. Biologic Response Modifiers, DMARDs: Etanercept, Infliximab Glucocorticoids, such as beclomethasone, methylprednisolone, betamethasone, prednisone, dexamethasone, and hydrocortisone

Pharmacologic Classifications of Treatment for Diabetes Mellitus

1. Sulfonylureas: Tolbutamide, Tolazamide, Glyburide, Glipizide
2. Biguanides: Metformin
3. Miscellaneous oral agents: Acarbose, Troglitazone
4. Insulin

Pharmacologic Classifications of Treatment for Alzheimer's Disease

1. Cholinesterase Inhibitor: Tacrine, Donepezil
2. Antipsychotics: Haloperidol, Thioridazine
3. Antidepressants: Desipramine, Fluoxetine, Trazodone, Paroxetine
4. Anticonvulsants: Carbamazepine, Valproic acid

In an embodiment, the compositions of the present invention may comprise a therapeutically effective amount of a RAGE fusion protein in combination with a single or multiple additional therapeutic agents. In addition to the agents heretofore described, the following therapeutic agents may be used in combination with the RAGE fusion proteins of the

present invention: immunosuppressants, such as cyclosporin, tacrolimus, rapamycin and other FK-506 type immunosuppressants.

In one embodiment, the present invention may therefore provide a method of treating RAGE mediated diseases, the method comprising administering to a subject in need thereof, a therapeutically effective amount of a RAGE fusion protein in combination with therapeutic agents selected from the group consisting of alkylating agents, antimetabolites, plant alkaloids, antibiotics, hormones, biologic response modifiers, analgesics, NSAIDs, DMARDs, biologic response modifiers (e.g., glucocorticoids), sulfonylureas, biguanides, insulin, cholinesterase inhibitors, antipsychotics, antidepressants, anticonvulsants, and immunosuppressants, such as cyclosporin, tacrolimus, rapamycin and other FK-506 type immunosuppressants. In a further embodiment, the present invention provides the pharmaceutical composition of the invention as described above, further comprising one or more therapeutic agents selected from the group consisting of alkylating agents, antimetabolites, plant alkaloids, antibiotics, hormones, biologic response modifiers, analgesics, NSAIDs, DMARDs, biologic response modifiers (e.g., glucocorticoids), sulfonylureas, biguanides, insulin, cholinesterase inhibitors, antipsychotics, antidepressants, anticonvulsants, and immunosuppressants, such as cyclosporin, tacrolimus, rapamycin and other FK-506 type immunosuppressants.

Lyophilized Formulations

In other embodiments, the present invention also provides formulations comprising a RAGE fusion protein. Embodiments of the formulations may comprise a lyophilized mixture of a lyoprotectant, a RAGE fusion protein, and buffer.

A variety of lyoprotectants may be used in the lyophilized RAGE fusion protein formulations of the present invention. In some embodiments, the lyoprotectant may comprise a non-reducing sugar. For example, the non-reducing sugar may comprise sucrose, mannitol, or trehalose. Also, a variety of buffers may be used in the lyophilized RAGE fusion protein formulation. In certain embodiments, the buffer may comprise histidine.

The lyophilized RAGE fusion protein may comprise additional components. In certain embodiments, the RAGE fusion protein formulation may further comprise at least one of a surfactant, a chelating agent or a bulking agent. In one embodiment, the reconstituted RAGE fusion protein formulation comprises about 40-100 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 2 mM to about 50 mM histidine; about 60 mM to about 65 mM sucrose; about 0.001% to about 0.05% Tween 80; and a pH of about 6.0 to 6.5. For example, the reconstituted RAGE fusion protein formulation may, in certain embodiments, comprise about 40-50 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 10 mM histidine; about 65 mM sucrose; about 0.01% Tween 80; and at a pH of about 6.0. Or, other concentrations of the RAGE fusion protein may be used as further described herein.

In one embodiment, the present invention comprises a reconstituted formulation comprising a lyophilized RAGE fusion protein reconstituted in a diluent, wherein the RAGE fusion protein concentration in the reconstituted formulation is within the range from about 1 mg/mL to about 400 mg/mL. Or, other concentrations of the RAGE fusion protein may be used as described herein.

In other embodiments, the present invention may also comprise methods for preparing stable reconstituted formulation of a RAGE fusion protein. The reconstituted formulation may

comprise a concentration that is suitable for direct use (e.g., direct administration to a subject) or that may be further diluted and/or mixed with a delivery agent.

In certain embodiments, the method may comprise reconstituting a lyophilized mixture of the RAGE fusion protein and a lyoprotectant in a diluent such that the RAGE fusion protein concentration in the reconstituted formulation is in a range from about 1 mg/mL to about 400 mg/mL. Or, other concentrations as described herein may be used as described herein.

A variety of lyoprotectants may be used in the reconstituted RAGE fusion protein formulations of the present invention. In some embodiments, the lyoprotectant may comprise a non-reducing sugar. For example, the non-reducing sugar may comprise sucrose, mannitol, or trehalose. Also, a variety of buffers may be used in the lyophilized RAGE fusion protein formulation. In certain embodiments, the buffer may comprise histidine.

The reconstituted RAGE fusion protein formulation may comprise additional components. In certain embodiments, the RAGE fusion protein formulation may further comprise at least one of a surfactant, a chelating agent or a bulking agent. In one embodiment, the reconstituted RAGE fusion protein formulation comprises about 40-100 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 2 mM to about 50 mM histidine; about 60 mM to about 65 mM sucrose; about 0.001% to about 0.05% Tween 80; and a pH of about 6.0 to 6.5. For example, the reconstituted RAGE fusion protein formulation may, in certain embodiments, comprise about 40-50 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 10 mM histidine, about 65 mM sucrose, about 0.01% Tween 80, and at a pH of about 6.0.

A variety of diluents suitable for pharmaceuticals may be used to reconstitute the lyophilized RAGE fusion protein. In an embodiment, the lyophilized RAGE fusion protein is sterile. Also in an embodiment, the diluent is sterile. In one embodiment, the diluent may comprise water for injection (WFI). Also, in certain embodiments, the amount of diluent added is based on the therapeutic dosage and the pharmacokinetic profile of the RAGE fusion protein, as well as the biocompatibility of the formulation and carrier being administered. In an embodiment, the reconstituted formulation is isotonic.

The RAGE fusion protein formulation may comprise a stable therapeutic agent that is formulated for use in a clinic or as a prescription medicine. For example, in certain embodiments, the reconstituted RAGE fusion protein formulation may exhibit less than 10%, or less than 5%, or less than 3% decomposition after one week at 40 degrees Centigrade.

Also, the RAGE fusion protein formulation may be stable upon reconstitution in a diluent. In certain embodiments, less than about 10%, or about 5%, or about 4%, or about 3%, or about 2%, or about 1% of the RAGE fusion protein is present as an aggregate in the RAGE fusion protein formulation.

The reconstituted RAGE fusion protein formulation may be suitable for administration by various routes and as is required for treatment of the RAGE-mediated disorder of interest. In certain embodiments, the reconstituted RAGE fusion protein formulation is suitable for at least one of intravenous, intraperitoneal, or subcutaneous administration of the formulation to a subject.

For example, in certain embodiments, the present invention may comprise a stable reconstituted formulation comprising a RAGE fusion protein in a concentration of at least 10 mg/mL, or at least 20 mg/mL, or at least 50 mg/mL and a

diluent, where the reconstituted formulation has been prepared from a lyophilized mixture of the RAGE fusion protein and a lyoprotectant. In alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation may be at least 100 mg/mL, or at least 200 mg/mL, or at least 400 mg/mL. In yet alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation is in an amount within the range of about 0.5 mg/mL to about 400 mg/mL, or about 1 mg/mL to about 200 mg/mL, or about 40 mg/mL to about 400 mg/mL, about 40 to 100 mg/mL, or about 40-50 mg/mL. The formulation may also comprise a buffer.

In yet other embodiments, the present invention may comprise articles of manufacture that include RAGE fusion proteins. For example, in certain embodiments, the article of manufacture may comprise a container which holds a lyophilized RAGE fusion protein, and instructions for reconstituting the lyophilized formulation with a diluent. In certain embodiments, the articles of manufacture may comprise a container which holds a formulation comprising a lyophilized mixture of a lyoprotectant, a RAGE fusion protein, and buffer. The article of manufacture may also comprise instructions for reconstituting the lyophilized formulation with a diluent.

A variety of lyoprotectants may be used in the articles of manufacture of the present invention. In some embodiments, the lyoprotectant may comprise a non-reducing sugar. For example, the non-reducing sugar may comprise sucrose, mannitol, or trehalose. Also, a variety of buffers may be used in the lyophilized RAGE fusion protein formulation. In certain embodiments, the buffer may comprise histidine.

The RAGE fusion protein formulation of the articles of manufacture of the present invention may comprise additional components. In certain embodiments, the lyophilized RAGE fusion protein formulation may further comprise at least one of a surfactant, a chelating agent or a bulking agent. In one embodiment of the articles of manufacture of the present invention, upon reconstitution according to the instructions provided, the RAGE fusion protein formulation comprises about 40-100 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 2 mM to about 50 mM histidine; about 60 mM to about 65 mM sucrose; about 0.001% to about 0.05% Tween 80; and a pH of about 6.0 to 6.5. For example, the reconstituted RAGE fusion protein formulation may, in certain embodiments, comprise about 40-50 mg/mL RAGE fusion protein comprising the sequence as set forth in SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57; about 10 mM histidine; about 65 mM sucrose; about 0.01% Tween 80; and at a pH of about 6.0. Or other concentrations of the RAGE fusion protein may be used as described herein.

A variety of diluents suitable for pharmaceuticals may be provided for reconstituting the lyophilized RAGE fusion protein. In an embodiment, the lyophilized formulation is sterile. Alternatively or additionally, the diluent may be sterile. In one embodiment, the diluent may comprise water for injection (WFI). Thus, the article of manufacture may further comprise a second container which holds a diluent for reconstituting the lyophilized formulation, wherein the diluent is water for injection (WFI). In an embodiment, the reconstituted formulation is isotonic.

Also, in certain embodiments, the amount of diluent added is based on the therapeutic dosage and the pharmacokinetic profile of the RAGE fusion protein, as well as the biocompatibility of the formulation and carrier being administered. In alternate embodiment, the instructions are for reconstituting the lyophilized formulation so as to have the concentrations

as described herein. For example, in certain embodiments, the instructions are for reconstituting the lyophilized formulation such that the RAGE fusion protein concentration in the reconstituted formulation is within the range from about 40 mg/mL to about 100 mg/mL.

The RAGE fusion protein formulation provided as the article of manufacture may comprise a stable therapeutic agent that is formulated for use in a clinic or as a prescription medicine. For example, in certain embodiments, when reconstituted according to the instructions provided, the RAGE fusion protein may exhibit less than 10%, or less than 5%, or less than 3% decomposition after one week at 40 degrees Centigrade. Also, the RAGE fusion protein formulation may be stable upon reconstitution in a diluent. In certain embodiments, less than about 10%, or about 5%, or about 4%, or about 3%, or about 2%, or about 1% of the RAGE fusion protein is present as an aggregate in the RAGE fusion protein formulation.

Also, in certain embodiments, when reconstituted according to the instructions provided, the reconstituted RAGE fusion protein formulation may be suitable for administration by various routes and as is required for treatment of the RAGE-mediated disorder of interest. In certain embodiments, the reconstituted RAGE fusion protein formulation is suitable for at least one of intravenous, intraperitoneal, or subcutaneous administration of the formulation to the subject.

In certain embodiments of the formulations, articles of manufacture, and methods of making formulations comprising a RAGE fusion protein, the RAGE fusion protein concentration in the reconstituted formulation may be at least 10 mg/mL, or at least 20 mg/mL, or at least 50 mg/mL. In alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation may be at least 100 mg/mL, or 200 mg/mL, or 400 mg/mL. For example, in alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation is at least about 0.5 to 400 mg/mL, or about 1 to 200 mg/mL, 40 to 400 mg/mL, 50 to 400 mg/mL, 40 to 100 mg/mL, 50 to 100 mg/mL, or about 40-50 mg/mL. For example, in one embodiment, the RAGE fusion protein is administered in a formulation as a sterile aqueous solution having a pH that ranges from about 5.0 to about 6.5 and comprising from about 1 mg/mL to about 200 mg/mL of RAGE fusion protein, from about 1 millimolar to about 100 millimolar of histidine buffer, from about 0.01 mg/mL to about 10 mg/mL of polysorbate 80, from about 100 millimolar to about 400 millimolar of trehalose, and from about 0.01 millimolar to about 1.0 millimolar of disodium EDTA dihydrate.

Any of the embodiments described herein may be used as the RAGE fusion protein in the formulations of the present invention. Thus, for each of the lyophilized formulations, reconstituted lyophilized formulations, or the methods of making the lyophilized formulations or reconstituted lyophilized formulations, or the articles of manufacture comprising either the lyophilized formulations or the reconstituted lyophilized formulations of the present invention, the RAGE fusion protein may comprise a sequence derived from a RAGE ligand binding site linked to an immunoglobulin polypeptide.

Thus, embodiments of the RAGE fusion protein may comprise a RAGE polypeptide directly linked to a polypeptide comprising a C_H2 domain of an immunoglobulin or a portion of a C_H2 domain of an immunoglobulin as described herein. In certain embodiments, the RAGE polypeptide may comprise a RAGE interdomain linker linked to a RAGE immunoglobulin domain such that the C-terminal amino acid of the

RAGE immunoglobulin domain is linked to the N-terminal amino acid of the interdomain linker, and the C-terminal amino acid of the RAGE interdomain linker is directly linked to the N-terminal amino acid of a polypeptide comprising a C_H2 domain of an immunoglobulin, or a portion thereof. For example, certain embodiments of the fusion protein may comprise a first RAGE immunoglobulin domain and a first RAGE interdomain linker linked to a second RAGE immunoglobulin domain and a second RAGE interdomain linker, such that the N-terminal amino acid of the first interdomain linker is linked to the C-terminal amino acid of the first RAGE immunoglobulin domain, the N-terminal amino acid of the second RAGE immunoglobulin domain is linked to C-terminal amino acid of the first interdomain linker, the N-terminal amino acid of the second interdomain linker is linked to C-terminal amino acid of the second RAGE immunoglobulin domain, and the C-terminal amino acid of the RAGE second interdomain linker is directly linked to the N-terminal amino acid of the C_H2 immunoglobulin domain or a portion of a C_H2 domain of an immunoglobulin.

For example, in alternate embodiments, of the RAGE fusion protein, the RAGE polypeptide may comprise the amino acid sequence as set forth in SEQ ID NO: 10, or a sequence at least 90% identical thereto, or the amino acid sequence as set forth in SEQ ID NO: 47, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In other alternate embodiments, the RAGE fusion protein may comprise the amino acid sequence as set forth in at least one of SEQ ID NOs: 32, 33, 34, 35, 36, 37, 56, or 57, or a sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. For example, in certain embodiments, a sequence at least 90% identical to SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57 comprises the polypeptide of SEQ ID NOs: 32, 33, 34, 56, 35, 36, 37, or 57 without the C-terminal lysine.

Preparation of Lyophilized Formulations

In another embodiment, the present invention provides a pre-lyophilized formulation, a lyophilized formulation, a reconstituted formulation, and methods for preparation thereof.

After preparation of a RAGE fusion protein of interest as described above, a "pre-lyophilized formulation" may be produced. The amount of RAGE fusion protein present in the pre-lyophilized formulation may be determined taking into account the desired dose volumes, mode(s) of administration etc. In an embodiment, the amount of fusion protein in the pre-lyophilized formulation may be greater than 1 mg/mL. Also in certain embodiments, the amount of fusion protein in the pre-lyophilized formulation may be less than about 5 mg/mL, 10 mg/mL, 50 mg/mL, 100 mg/mL, or 200 mg/mL.

In a further embodiment, the pre-lyophilized formulation may be a pH-buffered solution at a pH from about 4-8. In another embodiment, the pre-lyophilized formulation may be a pH-buffered solution at a pH from about 5-7. In another embodiment, the pre-lyophilized formulation may be a pH-buffered solution at a pH of less than 6.7. In another embodiment, the pre-lyophilized formulation may be a pH-buffered solution at a pH of about 6.0. Exemplary buffers include histidine, phosphate, Tris, citrate, succinate and other organic acids as described herein. The buffer concentration may be from about 1 mM to about 100 mM, or less than about 50 mM, or from about 2 mM to about 50 mM, or less than about 15 mM, or from about 3 mM to about 15 mM depending, for example, on the buffer and the desired isotonicity of the formulation (e.g. of the reconstituted formulation). In an embodiment, the buffer is histidine.

The lyoprotectant may be added to the pre-lyophilized formulation. In an embodiment, the lyoprotectant comprises a sugar. In another embodiment, the lyoprotectant comprises a non-reducing sugar. In another embodiment, the lyoprotectant comprises the non-reducing sugar sucrose. Or, the non-reducing sugar may comprise mannitol. Or, the non-reducing sugar may comprise trehalose. The amount of lyoprotectant in the pre-lyophilized formulation is generally such that upon reconstitution, the resulting formulation will be isotonic. However, a hypertonic reconstituted formulation may also be suitable, for example in formulations for peripheral parenteral administration. In addition, the amount of lyoprotectant should not be so low such that an unacceptable amount of degradation and/or aggregation of the protein occurs upon lyophilization. In alternate embodiments, an unacceptable amount of aggregation may be where 20%, or 10%, or 5% or more of the RAGE fusion protein is present as an aggregate in a formulation. An exemplary range of lyoprotectant concentration in the pre-lyophilized formulation may be less than about 400 mM. In another embodiment, the range of lyoprotectant concentration in the pre-lyophilized formulation is less than about 100 mM. In alternate embodiments, the range of lyoprotectant concentration in the pre-lyophilized formulation may therefore range from about 0.5 mM to 400 mM, or from about 2 mM to 200 mM, or from about 30 mM to about 150 mM, or from about 60-65 mM. Also, in some embodiments, the lyoprotectant is added in an amount to render the reconstituted formulation isotonic.

The ratio of RAGE fusion protein to lyoprotectant in the pre-lyophilized formulation is selected for each RAGE fusion protein and lyoprotectant combination. In an embodiment of an isotonic reconstituted formulation with a high RAGE fusion protein concentration (e.g., greater than or equal to about 50 mg/mL), the molar ratio of lyoprotectant to RAGE fusion protein may be from about 50 to about 1500 moles lyoprotectant to 1 mole RAGE fusion protein. In another embodiment, the molar ratio of lyoprotectant to RAGE fusion protein may be from about 150 to about 1000 moles of lyoprotectant to 1 mole fusion protein. In another embodiment, the molar ratio of lyoprotectant to RAGE fusion protein may be from about 150 to about 300 moles of lyoprotectant to 1 mole RAGE fusion protein. For example, these ranges may be suitable where the lyoprotectant is a non-reducing sugar, such as sucrose, trehalose or mannitol.

In another embodiment of the invention, a surfactant may be added to the pre-lyophilized formulation. Alternatively, or in addition, the surfactant may be added to the lyophilized formulation and/or the reconstituted formulation. Exemplary surfactants include nonionic surfactants such as polysorbates (e.g. polysorbates 20 or 80) (Tween 20™ or Tween 80™); poloxamers (e.g. poloxamer 188). The amount of surfactant added is such that it reduces aggregation of the reconstituted protein and minimizes the formation of particulates after reconstitution. For example, the surfactant may be present in the pre-lyophilized formulation in an amount from about 0.001% to 0.5%. For example, in an embodiment where the surfactant comprises polysorbate 80, the surfactant may be present in the pre-lyophilized formulation in an amount from about 0.005% to 0.05%, or about 0.008% to 0.012%, or at about 0.01%. Alternatively, the surfactant may be present in the formulation so as to comprise a final concentration ranging from 0.001 mg/mL to about 100 mg/mL, or about 0.01 mg/mL to about 10 mg/mL.

In certain embodiments of the invention, a mixture of the lyoprotectant (such as sucrose or histidine) and a bulking agent (e.g. mannitol or glycine) may be used in the preparation of the pre-lyophilization formulation. The bulking agent

may allow for the production of a uniform lyophilized cake without excessive pockets therein etc.

Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the pre-lyophilized formulation (and/or the lyophilized formulation and/or the reconstituted formulation) provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include additional buffering agents; preservatives; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g. Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counter ions such as sodium.

The RAGE fusion protein formulations of the present invention may also contain additional proteins as necessary for the particular indication being treated. The additional proteins may be selected such that the proteins each have complementary activities that do not adversely affect each other or the RAGE fusion protein. Such proteins are suitably present in combination in amounts that are effective for the purpose intended.

The RAGE fusion protein formulations of the present invention may be sterile for in vivo administration. This may be accomplished by filtration through sterile filtration membranes, prior to, or following, lyophilization and reconstitution.

After the RAGE fusion protein, lyoprotectant and other optional components are mixed together, the formulation may be lyophilized. Many different freeze-dryers are available for this purpose such as Hull50™ (Hull, USA) or GT20™ (Leybold-Heraeus, Germany) freeze-dryers. Freeze-drying is accomplished by freezing the formulation and subsequently subliming ice from the frozen content at a temperature suitable for primary drying. Under this condition, the product temperature is below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -50 to 25° C. (provided the product remains frozen during primary drying) at a suitable pressure, ranging typically from about 50 to 250 mTorr. In an embodiment, the pressure is about 100 mTorr and the sample may be lyophilized between about -30 and 25° C. The formulation, size and type of the container holding the sample (e.g., glass vial) and the volume of liquid may dictate the time required for drying, which can range from a few hours to several days (e.g., 40-60 hrs). Freeze-drying conditions can be varied depending on the formulation and vial size.

In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 2, 3, 5, 10, 20, 50, 100, or 250 cc vial. In an embodiment, the container is any container suitable to prepare a reconstituted formulation having a volume of less than or equal to 100 mL.

As a general proposition, lyophilization will result in a lyophilized formulation in which the moisture content thereof is less than about 5%. In an embodiment, the moisture content of the lyophilized formulation is less than about 3%. In another embodiment, the moisture content of the lyophilized formulation is less than about 1%.

Reconstitution of the Lyophilized Formulation

At the desired stage, typically when it is time to administer the RAGE fusion protein to a patient or subject, the lyophilized formulation may be reconstituted with a diluent such

that the RAGE fusion protein concentration in the reconstituted formulation is about greater than 10 mg/mL, or greater than 20 mg/mL, or greater than 50 mg/mL, or about 30-50 mg/mL, or about 50 mg/mL. In alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation may be at least 100 mg/mL, or 200 mg/mL, or 400 mg/mL. For example, in alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation may be in the range of from about 1 mg/mL to about 600 mg/mL, or from about 1 mg/mL to about 500 mg/mL, or from about 1 mg/mL to about 400 mg/mL, or from about 1 mg/mL to about 200 mg/mL, or from about 10 mg/mL to about 400 mg/mL, or from about 10 mg/mL to about 200 mg/mL, or from about 40 mg/mL to about 400 mg/mL, or from about 40 mg/mL to about 200 mg/mL, or from about 50 mg/mL to about 400 mg/mL, or from about 50 mg/mL to about 200 mg/mL. In other embodiments, the RAGE fusion protein concentration in the reconstituted formulation is from about 40 mg/mL to about 100 mg/mL, or about 50 mg/mL to about 100 mg/mL, or about 40 mg/mL to about 50 mg/mL. Such RAGE fusion protein concentrations in the reconstituted formulation are considered to be particularly useful where subcutaneous delivery of the reconstituted formulation is intended. However, for other routes of administration, such as intravenous administration, lower concentrations of the protein in the reconstituted formulation may be desired (for example from about 5-50 mg/mL, or from about 10-40 mg/mL RAGE fusion protein in the reconstituted formulation). Thus, in some embodiments, the concentration of fusion protein in the reconstituted formulation may be the same or less than 2 times the concentration of the fusion protein in the pre-lyophilized formulation.

In certain embodiments, the fusion protein concentration in the reconstituted formulation is significantly higher than that in the pre-lyophilized formulation. For example, the fusion protein concentration in the reconstituted formulation may, in certain embodiments, be about 2-40, or 2-10, or 3-8 times that of the pre-lyophilized formulation. In an embodiment, the RAGE fusion protein concentration in the reconstituted formulation may be about 3-6 times that of the pre-lyophilized formulation. In another embodiment where the concentration of RAGE fusion protein in the pre-lyophilized formulation is about 15 mg/mL, the concentration of the RAGE fusion protein in the reconstituted formulation is greater than or equal to about 50 mg/mL (e.g., at least three fold or at least four fold greater).

The delivery of a high protein concentration is often advantageous or required for subcutaneous administration due to the volume limitations (less than or equal to 1.5 mL) and dosing requirements (greater than or equal to 100 mg). However, protein concentrations (greater than or equal to 50 mg/mL) may be difficult to achieve in the manufacturing process since at high concentrations, a protein may have a tendency to aggregate during processing and become difficult to manipulate (e.g. pump) and sterile filter. Alternatively, the lyophilization process may provide a method to allow concentration of a protein. For example, a RAGE fusion protein may be filled into vials at a volume (V_f) and then lyophilized. The lyophilized RAGE fusion protein is then reconstituted with a smaller volume (V_r) of water or preservative (e.g. BWFI) than the original volume (e.g. $V_r=0.25V_f$) resulting in a higher RAGE fusion protein concentration in the reconstituted solution. This process also results in the concentration of the buffers and excipients. For subcutaneous administration, the solution is desirably isotonic.

Reconstitution generally takes place at a temperature of about 25° C. to ensure complete hydration, although other

temperatures may be employed as desired. The time required for reconstitution may depend, e.g., on the type of diluent, amount of excipient(s) and protein. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. In an embodiment, the diluent provides a reconstituted formulation suitable for injection. In another embodiment, where the diluent provides a reconstituted formulation suitable for injection, the diluent comprises water for injection (WFI). The diluent optionally contains a preservative. The amount of preservative employed may be determined by assessing different preservative concentrations for compatibility with the protein and preservative efficacy testing.

In alternate embodiments, the reconstituted formulation may have less than 8,000, or less than 6,000, or less than 4,000, or less than 2,000, or less than 1,000, or less than 600, or less than 400, or less than 200 or less than 100, or less than 50 particles that are equal to or greater than 10 μ m in size per 50 mL container. In other embodiments, the reconstituted formulation may have less than 8,000, or less than 6,000, or less than 4,000, or less than 2,000, or less than 1,000, or less than 600, or less than 400, or less than 200 or less than 100, or less than 50 particles that are equal to or greater than 25 μ m in size per 50 mL container.

Administration of the Reconstituted Formulation

The reconstituted formulation may be administered to a mammal in need of treatment with the RAGE fusion protein, such as a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intraarticular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

In embodiments, the reconstituted formulation may be administered to the mammal by subcutaneous (i.e. beneath the skin) administration. For such purposes, the reconstituted formulation may be injected using a syringe. However, other devices for administration of the reconstituted formulation are available such as injection devices (e.g. the Inject-ease™ and Genject™ devices); injector pens (such as the GenPen™); needleless devices (e.g. MediJector™ and BioJector™); and subcutaneous patch delivery systems.

The appropriate dosage or therapeutically effective amount of the RAGE fusion protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the RAGE fusion protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the RAGE fusion protein, and the discretion of the attending physician. The RAGE fusion protein may be administered to the subject (e.g., patient) at one time or over a series of treatments or may be administered to the patient at any time from diagnosis onwards. The RAGE fusion protein may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question. In an embodiment, a dosage from about 0.1-20 mg/kg is an initial candidate dosage for administration to the subject, whether, for example, by one or more separate administrations. As described above, other dosage regimens may be useful.

Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the lyophilized formulation of the present invention and provides instructions for its reconstitution and/or use. The article of manufacture may comprise a container. Suitable containers include, for example, bottles, vials (e.g. dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container

may be formed from a variety of materials such as glass or plastic. The container may hold the lyophilized formulation. In certain embodiments, there may be a label affixed to, or associated with, the container. The label may indicate instructions for reconstitution and/or use. For example, in certain 5 embodiments, the label may indicate that the lyophilized formulation is reconstituted to protein concentrations as described above. The label may further indicate that the formulation is useful or intended for subcutaneous administration.

The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g., from 2-6, or 2-10, or 2-50 administrations) of the reconstituted formulation. The article of manufacture may further comprise a second container comprising a suitable diluent (e.g. WFI). Upon mixing of the diluent and the lyophilized formulation, the final RAGE fusion protein concentration in the reconstituted formulation will generally be at least 10 mg/mL. In one embodiment, the final RAGE fusion protein concentration in the reconstituted formulation is at least about 20 mg/mL. In another embodiment, the final RAGE fusion protein concentration in the reconstituted formulation is at least about 50 mg/mL. In alternate embodiments, the RAGE fusion protein concentration in the reconstituted formulation may be at least 100 mg/mL, or 200 mg/mL, or 400 mg/mL. In other embodiments, the final RAGE fusion protein concentration on the reconstituted formulation is between about 1-400 mg/mL, or 1-200 mg/mL, or 1-100 mg/mL, or 10-400 mg/mL, or 10-200 mg/mL, or 10-100 mg/mL, or from 40-400 mg/mL, or from 40-200 mg/mL, or from 40-100 mg/mL, or from 50-400 mg/mL, or from 50-200 mg/mL, or from 50-100 mg/mL, or from 40 mg/mL to 50 mg/mL. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

EXAMPLES

Features and advantages of the inventive concept covered by the present invention are further illustrated in the examples which follow.

Example 1A

Production of RAGE Fusion Proteins

Two plasmids were constructed to express RAGE-IgG fusion proteins. Both plasmids were constructed by ligating different lengths of a 5' cDNA sequence from human RAGE with the same 3' cDNA sequence from human IgG Fc (γ 1). These expression sequences (i.e., ligation products) were then inserted in pcDNA3.1 expression vector (Invitrogen, Calif.). The nucleic acid sequences that encode the RAGE fusion protein coding region are shown in FIGS. 2 and 3. For TTP-4000 RAGE fusion protein, the nucleic acid sequence from 1 to 753 (highlighted in bold) encodes the RAGE N-terminal protein sequence, whereas the nucleic acid sequence from 754 to 1386 encodes the IgG Fc protein sequence without the hinge (FIG. 2). For TTP-3000, the nucleic acid sequence from 1 to 408 (highlighted in bold) encodes the RAGE N-terminal protein sequence, whereas the nucleic acid sequence from 409 to 1041 encodes the IgG Fc protein sequence without the hinge (FIG. 3).

To produce the RAGE fusion proteins, the expression vectors comprising the nucleic acid sequences of either SEQ ID NO: 30 or SEQ ID NO: 31 were stably transfected into CHO

cells. Positive transformants were selected for neomycin resistance conferred by the plasmid and cloned. High producing clones as detected by Western Blot analysis of supernatant were expanded and the gene product was purified by affinity chromatography using Protein A columns. Expression was optimized so that cells were producing recombinant TTP-4000 at levels of about 1.3 grams per liter.

Example 1B

Alternate Production of Four Domain RAGE Fusion Proteins

A plasmid was constructed to express RAGE-IgG fusion proteins. The plasmid was constructed by ligating a 5' cDNA sequence from human RAGE with a 3' cDNA sequence from human IgG Fc(γ 1) without the Fc hinge region. PCR was used to amplify the cDNA. Further, on the 5' end, the PCR primer added an Eco RI restriction enzyme site from cloning and a Kozak consensus translation initiation sequence. On the 3' end, the PCR primer added a Xho I restriction just past the terminal codon. On the 3' end, the PCR primer also included two silent base changes that remove a cryptic RNA splice site in the immunoglobulin portion near the terminal codon. The codon encoding for proline (residue 459 based on numbering in the protein sequence in SEQ ID NO: 32) was changed from CCG to CCC, and the codon encoding for glycine (residue 460 based on numbering in the protein sequence in SEQ ID NO: 32) was changed from GGT to GGG. The PCR fragment was digested with Eco RI and Xho I and then inserted into a retrovector plasmid (pCNS-newMCS-WPRE (new ori), available from Gala, Inc.) that had been digested with Mfe I (to form a compatible end With Eco RI) and digested with Xho I. The inserted portion of the cloned plasmid and cloning junctions were sequenced to ensure that no mutations occurred during cloning.

To produce the RAGE-IgG fusion protein, the expression vector comprising the nucleic acid sequence SEQ ID NO: 54 was stably transfected in CHO cells.

The sequence of the isolated RAGE fusion protein TTP-4000 expressed by the transfected cells was confirmed by various characterization studies as either SEQ ID NO: 34 or SEQ ID NO: 56, or both SEQ ID NO: 34 and SEQ ID NO: 56. Thus, the signal sequence encoded by the first 23 amino acids of SEQ ID NO: 32 was cleaved and the N-terminal residue was glutamine (Q) or pyroglutamic acid (pE) or a mixture thereof. Characterization studies also showed glycosylation sites at N2 and N288 (based on numbering of SEQ ID NO: 34 or SEQ ID NO: 56) and showed that the C_H3 region of the RAGE fusion protein may have its C-terminal residue cleaved off through a post-translational modification when expressed in this recombinant system.

Example 1C

Alternate Production of Three Domain RAGE Fusion Proteins

A plasmid can be constructed to express three domain RAGE-IgG fusion proteins (e.g., one RAGE domain and two IgG domains) such as TTP-3000 in the manner described above for TTP-4000. The plasmid is constructed by ligating a 5' cDNA sequence from human RAGE encoding amino acids 1-136 of human RAGE with a 3' cDNA sequence from human IgG Fc(γ 1) without the Fc hinge region. PCR can be used to amplify the cDNA. On the 5' end, a PCR primer may add a restriction site (e.g., an Eco RI restriction enzyme site as used

for TTP-4000) for cloning and a Kozak consensus translation initiation sequence. On the 3' end, the PCR primer may also add a restriction site (e.g., a Xho I restriction site) just past the terminal codon. The PCR primers may also include silent base changes as may be needed to remove any cryptic RNA splice sites, such as the cryptic RNA splice sites located at the 3' end of the immunoglobulin C_H2 domain as describes in Example 1B. To remove these cryptic splice sites, the codon encoding for proline 344 of SEQ ID NO: 35 (i.e., residues 1030-1032 based on numbering in the DNA sequence in SEQ ID NO: 31) may be changed from CCG to CCC, and the codon encoding for glycine 345 of SEQ ID NO: 35 (residues 1033-1035 based on numbering in the DNA sequence in SEQ ID NO: 31) may be changed from GGT to GGG. The PCR fragment may then be digested with the appropriate restriction enzymes (e.g., Eco RI and Xho I), and inserted into the retrovector plasmid pCNS-newMCS-WPRE (new ori; available from Gala, Inc.). The vector may be digested with Mfe I to form a compatible end with Eco RI, and also digested with Xho I. The inserted portion of the cloned plasmid and cloning junctions can be sequenced to ensure that no mutations occurred during cloning.

To produce the RAGE-IgG fusion protein, the expression vector comprising the nucleic acid sequence SEQ ID NO: 55 (i.e., comprising the change in DNA sequence to remove cryptic splice sites) can be stably transfected in CHO cells as described in Example 1A and 1B.

The sequence of the isolated RAGE fusion protein TTP-3000 expressed by the transfected cells may be either SEQ ID NO: 36, SEQ ID NO: 37 or SEQ ID NO: 57, or a combination of SEQ ID NO: 36, SEQ ID NO: 37 and/or SEQ ID NO: 57. Thus, the signal sequence encoded by the first 22 and/or 23 amino acids of SEQ ID NO: 35 may be cleaved and the N-terminal residue may be glutamine (Q) or pyroglutamic acid (pE) or a mixture thereof. Glycosylation may occur at sites at N2 and N174 (based on numbering of SEQ ID NO: 37 or SEQ ID NO: 57) and/or other glycosylation sites that may be present. The C_H3 region of the RAGE fusion protein may have its C-terminal residue cleaved off through a post-translational modification when expressed in this recombinant system.

Example 2

Method for Testing Activity of a RAGE-IgG1 Fusion Protein

A. In vitro Ligand Binding:

Known RAGE ligands were coated onto the surface of Maxisorb plates at a concentration of 5 micrograms per well. Plates were incubated at 4° C. overnight. Following ligand incubation, plates were aspirated and a blocking buffer of 1% BSA in 50 mM imidazole buffer (pH 7.2) was added to the plates for 1 hour at room temperature. The plates were then aspirated and/or washed with wash buffer (20 mM Imidazole, 150 mM NaCl, 0.05% Tween-20, 5 mM CaCl₂ and 5mM MgCl₂, pH 7.2). A solution of TTP-3000 (TT3) at an initial concentration of 1.082 mg/mL and a solution of TTP-4000 (TT4) at an initial concentration of 370 µg/mL were prepared. The RAGE fusion protein was added at increasing dilutions of the initial sample. The RAGE fusion protein was allowed to incubate with the immobilized ligand at 37° C. for one hour after which the plate was washed and assayed for binding of the RAGE fusion protein. Binding was detected by the addition of an immunodetection complex containing a monoclonal mouse anti-human IgG1 diluted 1:11,000 to a final assay concentration (FAC) of 21 ng/100 µL, a biotinylated

goat anti-mouse IgG diluted 1:500, to a FAC of 500 ng/µL, and an avidin-linked alkaline phosphatase. The complex was incubated with the immobilized RAGE fusion protein for one hour at room temperature after which the plate was washed and the alkaline phosphatase substrate para-nitrophenylphosphate (PNPP) was added. Binding of the complex to the immobilized RAGE fusion protein was quantified by measuring conversion of PNPP to para-nitrophenol (PNP) which was measured spectrophotometrically at 405 nm.

As illustrated in FIG. 7, the RAGE fusion proteins TTP-4000 (TT4) and TTP-3000 (TT3) specifically interact with known RAGE ligands amyloid-beta (Abeta), S100b (S100), and amphoterin (Ampho). In the absence of ligand, i.e., BSA coating alone (BSA or BSA+wash) there was no increase in absorbance over levels attributable to non-specific binding of the immunodetection complex. Where amyloid beta is used as the labeled ligand it may be necessary to preincubate the amyloid beta before the assay. Preincubation may allow the amyloid beta to self-aggregate into pleated sheet form, as amyloid beta may preferentially bind to RAGE in the form of a pleated sheet.

Additional evidence for a specific interaction between RAGE fusion proteins TTP-4000 and TTP-3000 with RAGE ligands is exemplified in studies showing that a RAGE ligand is able to effectively compete with a known RAGE ligand for binding to the RAGE fusion proteins. In these studies, amyloid-beta (A-beta) was immobilized on a Maxisorb plate and RAGE fusion protein added as described above. In addition, a RAGE ligand was added to some of the wells at the same time as the RAGE fusion protein.

It was found that the RAGE ligand could block binding of TTP-4000 (TT4) by about 25% to 30% where TTP-4000 was present at 123 µg/mL (1:3 dilution, FIG. 8). When the initial solution of TTP-4000 was diluted by a factor of 10 or 30 (1:10 or 1:30), binding of the RAGE fusion protein to the immobilized ligand was completely inhibited by the RAGE ligand. Similarly, the RAGE ligand blocked binding of TTP-3000 (TT3) by about 50% where TTP-3000 was present at 360 µg/mL (1:3 dilution, FIG. 9). When the initial solution of TTP-3000 was diluted by a factor of 10 (1:10), binding of the RAGE fusion protein to the immobilized ligand was completely inhibited by the RAGE ligand. Thus, specificity of binding of the RAGE fusion protein to the RAGE ligand was dose dependent. Also, as shown in FIGS. 8 and 9, there was essentially no binding detected in the absence of RAGE fusion protein, i.e., using only the immunodetection complex ("Complex alone").

B. Effect of RAGE Fusion Proteins in a Cell Based Assays

Previous work has shown that the myeloid THP-1 cells may secrete TNF-α in response to RAGE ligands. In this assay, THP-1 cells were cultured in RPMI-1640 media supplemented with 10% FBS using a protocol provided by ATCC. The cells were induced to secrete TNF-α via stimulation of RAGE with 0.1 mg/ml S100b both in the absence and the presence of the RAGE fusion proteins TTP-3000 (TT3) or TTP-4000 (TT4) (10 µg), sRAGE (10 µg), and a human IgG (10 µg) (i.e., as a negative control). The amount of TNF-α secreted by the THP-1 cells was measured 24 hours after the addition of the proteins to the cell culture using a commercially available ELISA kit for TNF-α (R&D Systems, Minneapolis, Minn.). The results in FIG. 10 demonstrate that the RAGE fusion proteins inhibit the S100b/RAGE-induced production of TNF-α in these cells. As shown in FIG. 10, upon addition of 10 µg TTP-3000 or TTP-4000 RAGE fusion protein, induction of TNF-α by S100b (0.1 mg/ml FAC) was reduced by about 45% to 70%, respectively. Fusion protein TTP-4000 may be at least as effective in block-

61

ing S100b induction of TNF- α as is sRAGE (FIG. 10). Specificity of the inhibition for the RAGE sequences of TTP-4000 and TTP-3000 is shown by the experiment in which IgG alone was added to S100b stimulated cells. Addition of IgG and S100b to the assay shows the same levels of TNF- α as S100b alone. Specificity of the inhibition of TNF- α induction by TTP-4000 and TTP-3000 for RAGE sequences of the RAGE fusion protein is shown by an experiment in which IgG alone was added to S100b stimulated cells. It can be seen that the addition of IgG, i.e., human IgG without the RAGE sequence (Sigma human IgG added at 10 μ g/well), and S100b to the assay shows the same levels of TNF- α as S100b alone.

In another cell-based assay, the ability of TTP-4000 to prevent the RAGE ligand HMGB1 from interacting with RAGE and other HMGB1 receptors was evaluated. Unlike anti-RAGE antibodies that bind to RAGE and to prevent the interaction of a RAGE ligand with RAGE, TTP-4000 may block the interaction of a RAGE ligand with RAGE by binding to the RAGE ligand. HMGB 1 has been reported to be a ligand for RAGE and the Toll-Like Receptors 2 and 4 (Park et al., *J. Biol Chem.*, 2004; 279(9):7370-7). All three of these receptors (RAGE, Toll-like receptor 2, and Toll-like receptor 4) are expressed on THP-1 cells (Parker, et al., *J. Immunol.*, 2004,172(8):4977-86.).

In this experiment, THP-1 cells were stimulated to produce TNF- α by HMGB1 (50 mg/mL) in the presence or absence of either TTP4000 or anti-RAGE antibodies. Under the conditions used in the assay, HMGB1 should be the only inducer of TNF- α . The amount of TNF- α secreted by the THP-1 cells was measured 24 hours after the addition of the proteins to the cell culture using a commercially available ELISA kit for TNF- α (R&D Systems, Minneapolis, Minn.). The results in FIG. 11 demonstrate that the anti-RAGE antibody and RAGE fusion protein TTP-4000 block HMGB1 from interacting with RAGE expressed on the THP-1 cells, but that TTP-4000 inhibits HMGB 1-induced TNF- α production to a greater extent than does the anti-RAGE antibody. Thus, the data indicate that TTP-4000 may inhibit HMGB1 activity to a greater extent than anti-RAGE antibody by inhibiting HMGB1 from interacting with Toll-like receptors 2 and 4, as well as RAGE present on THP-1 cells.

Example 3

Pharmacokinetic Profile of TTP-4000

To determine whether TTP-4000 would have a superior pharmacokinetic profile as compared to human sRAGE, rats and nonhuman primates were given an intravenous (IV) injection of TTP-4000 (5 mg/kg) and then plasma was assessed for the presence of TTP-4000. In these experiments, two naive male monkeys received a single IV bolus dose of TTP-4000 (5 mg/ml/kg) in a peripheral vein followed by an approximate 1.0 milliliter (mL) saline flush. Blood samples (approximately 1.0 mL) were collected at pre-dose (i.e., prior to injection of the TTP-4000), or at 0.083, 0.25, 0.5, 2, 4, 8, 12, 24, 48, 72, 96, 120, 168, 240, 288, and 336 hours post dose into tubes containing (lithium heparin). Following collection, the tubes were placed on wet ice (maximum 30 minutes) until centrifugation under refrigeration (at 2 to 8 $^{\circ}$ C.) at 1500 \times g for 15 minutes. Each harvested plasma sample was then stored frozen (-70 $^{\circ}$ C. \pm 10 $^{\circ}$ C.) until assayed for RAGE polypeptide using an ELISA at various time-points following the injection, as described in Example 6.

The kinetic profile shown in FIG. 12 reveals that once TTP-4000 has saturated its ligands as evidenced by the fairly steep slope of the alpha phase in 2 animals, it retains a termi-

62

nal half-life of greater than 300 hours. This half-life is significantly greater than the half-life of human sRAGE in plasma (generally about 2 hours) and provides an opportunity for single injections for acute and semi-chronic indications. In FIG. 12 each curve represents a different animal under the same experimental conditions.

Example 4

TTP-4000 Fc Activation

Experiments were performed to measure the activation of the Fc receptor by RAGE fusion protein TTP-4000 as compared to human IgG. Fc receptor activation was measured by measuring TNF- α secretion from THP-1 cells that express the Fc receptor. In these experiments, a 96 well plate was coated with 10 μ g/well TTP-4000 or human IgG. Fc stimulation results in TNF- α secretion. The amount of TNF- α was measured by an Enzyme Linked Immunoabsorbent Assay (ELISA).

Thus, in this assay, the myeloid cell line, THP-1 (ATTC #TIB-202) was maintained in RPMI-1640 media supplemented with 10% fetal bovine serum per ATCC instructions. Typically, 40,000-80,000 cells per well were induced to secrete TNF-alpha via Fc receptor stimulation by precoating the well with 10 ug/well of either heat aggregated (63 $^{\circ}$ C. for 30 min) TTP-4000 or human IgG1. The amount of TNF-alpha secreted by the THP-1 cells was measured in supernatants collected from 24 hours cultures of cells in the treated wells using a commercially available TNF ELISA kit (R&D Systems, Minneapolis, Minn. #DTA00C) per instructions. Results are shown in

FIG. 13 where it can be seen that TTP-4000 generates less than 2 ng/well TNF and IgG generated greater than 40 ng/well.

Example 5

In vivo Activity of TTP-4000

The activity of TTP-4000 was compared to sRAGE in several in vivo models of human disease.

A. TTP-4000 in an Animal Model of Restenosis

The RAGE fusion protein TTP-4000 was evaluated in a diabetic rat model of restenosis which involved measuring smooth muscle proliferation and intimal expansion 21 days following vascular injury. In these experiments, balloon injury of left common carotid artery was performed in Zucker diabetic and nondiabetic rats using standard procedure. A loading dose (3mg/rat) of IgG, TTP-4000 or phosphate buffered saline (PBS) was administered intraperitoneally (IP) one day prior injury. A maintenance dose was delivered every other day until day 7 after injury (i.e., at day 1, 3, 5 and 7 after injury). The maintenance dose was high=1 mg/animal for one group, or low=0.3 mg/animal for the second group. To measure vascular smooth muscle cell (VSMC) proliferation, animals were sacrificed at 4 days and 21 days after injury.

For the measurement of cell proliferation, 4 day animals received intraperitoneal injection of bromodeoxyuridine (BrdU) 50 mg/kg at 18, 12, and 2 hours before euthanasia. After sacrifice, the entire left and right carotid arteries were harvested. Specimens were stored in Histochoice for at least 24 hours before embedding. Assessment of VSMC proliferation was performed using mouse anti-BrdU monoclonal antibody. A fluorescence labeled goat anti-mouse secondary anti-

body was applied. The number of BrdU-positive nuclei per section were counted by two observers blinded to the treatment regimens.

The remaining rats were sacrificed at 21 days for morphometric analysis. Morphometric analyses were performed by an observer blinded to the study groups, using computerized digital microscopic planimetry software Image-Pro Plus on serial sections, (5 mm apart) carotid arteries stained by Van Gieson staining. All data were expressed as mean \pm SD. Statistical analysis was performed with use of SPSS software. Continuous variables were compared using unpaired t tests. A values of $P \leq 0.05$ was considered to be statistically significant.

As seen in FIGS. 14A and 14B, TTP-4000 treatment significantly reduced the intima/media ratio and vascular smooth muscle cell proliferation in a dose-responsive fashion. In FIG. 14B, the y-axis represents the number of BrdU proliferating cells.

B. TTP4000 in an Animal Model of AD

Experiments were performed to evaluate whether TTP-4000 could affect amyloid formation and cognitive dysfunction in a mouse model of AD. The experiments utilized transgenic mice expressing the human Swedish mutant amyloid precursor protein (APP) under the control of the PDGF-B chain promoter. Over time, these mice generate high levels of the RAGE ligand, amyloid beta ($A\beta$). Previously, sRAGE treatment for 3 months has been shown to reduce both amyloid plaque formation in the brain and the associated increase in inflammatory markers in this model.

The APP mice (male) used in this experiment were designed by microinjection of the human APP gene (with the Swedish and London mutations) into mouse eggs under the control of the platelet-derived growth factor B (PDGF-B) chain gene promoter. The mice were generated on a C57BL/6 background and were developed by Molecular Therapeutics Inc. Animals were fed ad libitum and maintained by brother sister mating. The mice generated from this construct develop amyloid deposits starting at 6 months of age. Animals were aged for 6 months and then maintained for 90 days and sacrificed for amyloid quantification.

APP transgenic mice were administered vehicle or TTP4000 every other day [qod (i.p.)] for 90 days starting at 6 months of age. At the end of the experiment, animals were sacrificed and examined for $A\beta$ plaque burden in the brain (i.e., plaque number). A 6-month control APP group was used to determine the baseline of amyloid deposits. In addition, at the end of the study, the animals were subjected to behavioral (Morris water maze) analysis. The investigators were blinded to the study compounds. Samples were given to the mice at 0.25 ml/mouse/every other day. In addition, one group of mice were given 200 ug/day of human sRAGE.

1. Amyloid Beta Deposition

For histological examination, the animals were anesthetized with an intraperitoneal injection (IP) of sodium pentobarbital (50 mg/kg). The animals were transcardially perfused with 4° C., phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed and placed in 4% paraformaldehyde over night. The brains were processed to paraffin and embedded. Ten serial 30- μ m thick sections through the brain were obtained. Sections were subjected to primary antibody overnight at 4° C. ($A\beta$ peptide antibody) in order to detect the amyloid deposits in the brain of the transgenic animals (Guo et al., *J. Neurosci.*, 22:5900-5909 (2002)). Sections were washed in Tris-buffered saline (TBS) and secondary antibody was added and incubated for 1 hour at room temperature. After washing, the sections were incubated as instructed in the Vector ABC Elite kit (Vector

Laboratories) and stained with diaminobenzoic acid (DAB). The reactions were stopped in water and cover-slipped after treatment with xylene. The amyloid area in each section was determined with a computer-assisted image analysis system, consisting of a Power Macintosh computer equipped with a Quick Capture frame grabber card, Hitachi CCD camera mounted on an Olympus microscope and camera stand. NIH Image Analysis Software, v. 1.55 was used. The images were captured and the total area of amyloid was determined over the ten sections. A single operator blinded to treatment status performed all measurements. Summing the amyloid volumes of the sections and dividing by the total number of sections was done to calculate the amyloid volume.

For quantitative analysis, an enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of human total $A\beta$, $A\beta_{total}$ and $A\beta_{1-42}$ in the brains of APP transgenic mice (Biosource International, Camarillo, Calif.). $A\beta_{total}$ and $A\beta_{1-42}$ were extracted from mouse brains by guanidine hydrochloride and quantified as described by the manufacturer. This assay extracts the total $A\beta$ peptide from the brain (both soluble and aggregated).

2. Cognitive Function

The Morris water-maze testing was performed as follows. All mice were tested once in the Morris water maze test at the end of the experiment. Mice were trained in a 1.2 m open field water maze. The pool was filled to a depth of 30 cm with water and maintained at 25° C. The escape platform (10 cm square) was placed 1 cm below the surface of the water. During the trials, the platform was removed from the pool. The cued test was carried out in the pool surrounded with white curtains to hide any extra-maze cues. All animals underwent non-spatial pretraining (NSP) for three consecutive days. These trials are to prepare the animals for the final behavioral test to determine the retention of memory to find the platform. These trials were not recorded, but were for training purposes only. For the training and learning studies, the curtains were removed to extra maze cues (this allowed for identification of animals with swimming impairments). On day 1, the mice were placed on the hidden platform for 20 seconds (trial 1), for trials 2-3 animals were released in the water at a distance of 10 cm from the cued-platform or hidden platform (trial 4) and allowed to swim to the platform. On the second day of trials, the hidden platform was moved randomly between the center of the pool or the center of each quadrant. The animals were released into the pool, randomly facing the wall and were allowed 60 seconds to reach the platform (3 trials). In the third trial, animals were given three trials, two with a hidden platform and one with a cued platform. Two days following the NSP, animals were subjected to final behavioral trials (Morris water maze test). For these trials (3 per animal), the platform was placed in the center of one quadrant of the pool and the animals released facing the wall in a random fashion. The animal was allowed to find the platform or swim for 60 seconds (latency period, the time it takes to find the platform). All animals were tested within 4-6 hours of dosing and were randomly selected for testing by an operator blinded to the test group.

The results are expressed as the mean \pm standard deviations (SD). The significance of differences in the amyloid and behavioral studies were analyzed using a t-test. Comparisons were made between the 6-month-old APP control group and the TTP-4000 treated animals, as well as, the 9-month-old APP vehicle treated group and the TTP-4000 treated animals. Differences below 0.05 were considered significant. Percent changes in amyloid and behavior were determined by taking the summation of the data in each group and dividing by the comparison (i.e., 1, i.p./6 month control=% change).

FIGS. 15A and 15B show that mice treated for 3 months with either TTP-4000 or mouse sRAGE had fewer A β plaques and less cognitive dysfunction than vehicle and negative control human IgG1 (IgG1) treated animals. This data indicates that TTP-4000 is effective in reducing AD pathology in a transgenic mouse model. It was also found that like sRAGE, TTP-4000 can reduce the inflammatory cytokines IL-1 and TNF- α (data not shown).

C. Efficacy of TTP-4000 in an Animal Model of Stroke

TTP-4000 was also compared to sRAGE in a disease relevant animal model of stroke. In this model, the middle carotid artery of a mouse was ligated for 1 hour followed by 23 hours of reperfusion at which point the mice were sacrificed and the area of the infarct in the brain was assessed. Mice were treated with sRAGE or TTP-4000 or control immunoglobulin just prior to reperfusion.

In these experiments, male C57BL/6 were injected with vehicle at 250 μ l/mouse or TTP test articles (TTP-3000, TTP-4000 at 250 μ l/mouse). Mice were injected intraperitoneally, 1 hour after the initiation of ischemia. Mice were subjected to one hour of cerebral ischemia followed by 24 hours of reperfusion. To induce ischemia, each mouse was anesthetized and body temperature was maintained at 36-37 $^{\circ}$ C. by external warming. The left common carotid artery (CCA) was exposed through a midline incision in the neck. A microsurgical clip was placed around the origin of the internal carotid artery (ICA). The distal end of the ECA was ligated with silk and transected. A 6-0 silk was tied loosely around the ECA stump. The fire-polished tip of a nylon suture was gently inserted into the ECA stump. The loop of the 6-0 silk was tightened around the stump and the nylon suture was advanced into and through the internal carotid artery (ICA), until it rested in the anterior cerebral artery, thereby occluding the anterior communicating and middle cerebral arteries. After the nylon suture had been in place for 1 hour, the animal was re-anesthetized, rectal temperature was recorded and the suture was removed and the incision closed.

Infarct volume was determined by anesthetizing the animals with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and then removing the brains. The brains were then sectioned into four 2-mm sections through the infarcted region and placed in 2% triphenyltetrazolium chloride (TTC) for 30 minutes. After, the sections were placed in 4% paraformaldehyde over night. The infarct area in each section was determined with a computer-assisted image analysis system, consisting of a Power Macintosh computer equipped with a Quick Capture frame grabber card, Hitachi CCD camera mounted on a camera stand. NIH Image Analysis Software, v. 1.55 was used. The images were captured and the total area of infarct was determined over the sections. A single operator blinded to treatment status performed all measurements. Summing the infarct volumes of the sections calculated the total infarct volume. The results are expressed as the mean \pm standard deviation (SD). The significance of difference in the infarct volume data was analyzed using a t-test.

As illustrated by the data in Table 2, TTP-4000 was more efficacious than sRAGE in limiting the area of infarct in these animals suggesting that TTP-4000, because of its better half-life in plasma, was able to maintain greater protection in these mice.

Example 6

Detection of RAGE Fusion Protein by ELISA

Initially, 50 μ L of the RAGE specific monoclonal antibody 1HB1011 at a concentration of 10 μ g/mL in 1 \times PBS pH 7.3 is

coated on plates via overnight incubation. When ready for use, plates are washed three times with 300 μ L of 1 \times Imidazole-Tween wash buffer and blocked with 1% BSA. The samples (diluted) and standard dilutions of known TTP-4000 dilutions are added at 100 μ L final volume. The samples are allowed to incubate at room temperature for one hour. After incubation, the plates are washed three times. A Goat Anti-human IgG1 1 (Sigma A3312) AP conjugate in 1 \times PBS with 1% BSA is added and allowed to incubate at room temperature for 1 hour. The plates are washed three times. Color was elucidated with paranitrophenylphosphate.

Example 7

Quantification of RAGE Ligand Binding to RAGE Fusion Protein

FIG. 16 shows saturation-binding curves with TTP-4000 to various immobilized known RAGE ligands. The ligands are immobilized on a microtiter plate and incubated in the presence of increasing concentrations of RAGE fusion protein from 0 to 360 nM. The RAGE fusion protein-ligand interaction is detected using a polyclonal antibody conjugated with alkaline phosphatase that is specific for the IgG portion of the fusion chimera. Relative Kds were calculated using Graphpad Prizm software and match with established literature values of RAGE-RAGE ligand values. HMG1B=Ampoterin, CML=Carboxymethyl Lysine, A beta=Amyloid beta 1-40.

Example 8

Use of RAGE Fusion Protein to Prevent Allogeneic Transplant Rejection

RAGE blockade may be expected to block allogeneic transplant rejection. These experiments explored whether blockade of ligand-RAGE interactions using a RAGE fusion protein of the invention would attenuate rejection of islet cells that had been transplanted from a healthy donor into a diabetic animal as measured by the length of time that the transplanted animals maintained a blood glucose level below a target concentration. As discussed herein, it was found that administration of a RAGE fusion protein (e.g., TTP-4000) to diabetic animals that had received islet cell transplants significantly delayed the recurrence of hyperglycemia and thus rejection of transplanted islet cells in two (allogeneic and syngeneic) animal models of transplant.

A. Allogeneic Islet Transplantation in Mice

The first set of experiments tested whether administration of a RAGE fusion protein (TTP-4000) would modulate the allogeneic rejection of transplanted islet cells and the recurrence of diabetes in a C57BL/6J (B6) mouse model of diabetes.

Animal Model of Diabetes

C57BL/6J (6-8 week old) (B6) mice were made diabetic by a single intravenous injection of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, Mo.) at 200 mg/kg.

BALB/cJ (6-8 week old) (BALB) mice served as donors for islet transplantation, thus providing an allo-mismatch for islet transplants.

Islet Isolation

Mice (BALB/c) were anesthetized with ketamine HCl/xylazine HCl solution (Sigma, St. Louis Mo.). After intraductal injection of 3 ml of cold Hank's balanced salt solution (HBSS, Gibco, Grand Island N.Y.) containing 1.5 mg/ml of collagenase P (Roche Diagnostics, Branchburg, N.J.), pancreata were surgically procured and digested at 37 $^{\circ}$ C. for 20

67

mins. Islets were washed with HBSS and purified by discontinuous gradient centrifugation using Polysucrose 400 (Cellgro, Herndon Va.) having four different densities (26%, 23%, 20%, and 11%). The tissue fragments at the interface of the 20% and 23% layers were collected, washed and resuspended in HBSS. Individual islets, free of attached acinar, vascular and ductal tissues were handpicked under an inverted microscope, yielding highly purified islets for transplantation.

Islet Transplantation

Streptozotocin-induced diabetic C57BL/6 (B6) mice received islet grafts within 2 days of the diagnosis of diabetes. BALB/cJ (6-8 week old) (BALB) mice served as donors for allogeneic islet transplantation. For transplantation, 500-600 freshly isolated islets (i.e., approximately 550 islet equivalents) from donor mice were picked up with an infusion set and transplanted into the subcapsular space of the right kidney of a recipient.

Treatment with Test Compounds

Test compounds were administered as soon as the islets were transplanted; administration continued for about 60 days, depending upon how the control animal was faring. Mice were injected with 0.25 ml of either phosphate buffered saline (PBS), TTP-4000 in PBS, or IgG in PBS according to the regimen below (Table 3).

TABLE 3

Administration of Test Compounds and/or Vehicle				
Test Group	Number of mice	Loading Dose	Maintenance Dose	Regimen
Untreated Control	8			
Control Vehicle (PBS)	8	0.25 ml/dose/mouse on day 1	0.25 ml/dose/mouse starting on day 2	Once every other day (QOD) × 60 days; IP
IgG	8	(300 µg)	(100 ug)	(100 ug)
TTP-4000	8	0.25 ml/dose/mouse on day 1	0.25 ml/dose/mouse starting on day 2	Once every other day (QOD) × 60 days; IP
		(300 µg)	(100 ug)	(100 ug)
TTP-4000	8	0.25 ml/dose/mouse on day 1	0.25 ml/dose/mouse starting on day 2	Once every other day (QOD) × 60 days; 1P
		(300 µg)	(30 ug)	(30 ug)
TTP-4000	8	0.25 ml/dose/mouse on day 1	0.25 ml/dose/mouse starting on day 2	Once every other day (QOD) × 60 days; IP

Monitoring of Islet Graft Function

Islet graft function was monitored by serial blood glucose measurements daily for the first 2 weeks after islet transplantation, followed by every other day thereafter. Reversal of diabetes was defined as blood a glucose level of less than 200 mg/dl on two consecutive measurements. Graft loss was determined when blood glucose exceeded 250 mg/dl on two consecutive measurements. The results are shown in Table 4.

TABLE 4

Effects Of TTP-4000 On Allograft Islet Transplant*					
TTP-4000 300 ug LD + 100 ug qod ip (Group 1)	PBS (Group 2)	TTP-4000 300 ug + 30 ug qod ip (Group 3)	IgG 300 ug LD + 100 ug qod ip (Group 4)	Untreated control	
14	9	13	8	9	
16	8	14	9	8	
13	10	12	10	9	
13	8	12	8	10	

68

TABLE 4-continued

Effects Of TTP-4000 On Allograft Islet Transplant*					
	TTP-4000 300 ug LD + 100 ug qod ip (Group 1)	PBS (Group 2)	TTP-4000 300 ug + 30 ug qod ip (Group 3)	IgG 300 ug LD + 100 ug qod ip (Group 4)	Untreated control
5	12	11	11	8	9
	16	8	11	8	8
10	15	8	8	9	11
	14	8	8	11	9
					7
					9
					8
					9
15	Mean 14.125	8.75	11.125	8.875	8.833333
	SD 1.457738	1.164965	2.167124	1.125992	1.029857
	n 8	8	8	8	12

*Values in Table 4 reflect the day of graft loss for each animal as defined by recurrence of increased blood glucose levels.

20 The effects of administering TTP-4000 on allograft rejection for BALB/c islets in B6 mice are shown as a Kaplan-Meier Cumulative Survival Plot in FIG. 17. It can be seen that there is an increase in the time before detection of graft failure for animals treated with TTP-4000 (Groups 1 and 3) as opposed to animals that are not treated at all (Control) or animals treated with the vehicle (PBS) or (human IgG1). Using a variety of statistical analyses (Mantel-Cox Logrank, Breslow-Gehan-Wilcoxon; Tarone-Ware, Peto-Peto-Wilcoxon; and Harrington-Fleming) the differences between the Control and TTP-4000 (Groups 1 and 3) were significant (Table 5).

TABLE 5

Statistical Method	Control vs Group 1 (TTP 4000)		Control vs Group 3 (TTP 4000)			
	Chi-Square	DF*	P-value	Chi-Square	DF	P-value
Logrank (Mantel-Cox)	18.777	1	<0.0001	7.662	1	0.0056
Breslow-Gehan-Wilcoxon	15.092	1	0.0001	4.904	1	0.0268
Tarone-Ware	16.830	1	<0.0001	6.212	1	0.0127
Peto-Peto-Wilcoxon	14.359	1	0.0002	4.315	1	0.0378
Harrington-Fleming (rho = 0.5)	16.830	1	<0.0001	6.212	1	0.0127

*Degrees of Freedom

B. Islet Transplantation in NOD-Mice as a Model of Autoimmune Disease

50 The second set of experiments tested whether administration RAGE fusion protein (i.e. TTP-4000 or TTP-3000) would modulate the course of recurrent diabetes in NOD mice, using a syngeneic NOD transplant model.

Animal Model of Diabetes

55 Spontaneous autoimmune non-obese diabetic mice (NOD/LtJ) (12-25 weeks old) served as recipients for islet cells, while young pre-diabetic NOD/LtJ mice (6-7 weeks old) served as donors in syngeneic islet transplantation. Islets for transplantation were isolated as described above in Section A (Allogeneic Islet Transplantation).

Islet transplantation:

65 Diabetic NOD/LtJ mice received islet grafts within 2 days of the diagnosis of diabetes. 500-600 freshly isolated islets (approximately 550 islet equivalents) from donor mice were picked up with an infusion set and transplanted into the subcapsular space of the right kidney.

69

Treatment with Test Compounds

Test compounds were administered as soon as the islets were transplanted and continued for approximately 8 weeks. Mice were injected with 0.25 ml of either PBS, TTP-4000 in PBS, or TTP-3000 in PBS according to the regimen below (Table 6).

TABLE 6

Group	No. mice	Loading	Maintenance	Regimen
		Dose Volume	Dose Volume	
TTP-4000	8	(300 µg) 0.25 ml/ dose/mouse on day 1	(100 µg) 0.25 ml/dose/ mouse starting on day 2	(100 µg) Once every other day (QOD) × 8 weeks; IP
TTP-3000	8	(300 µg) 0.25 ml/ dose/mouse on day 1	(100 µg) 0.25 ml/dose/ mouse starting on day 2	(100 µg) Once every other day (QOD) × 8 weeks; IP
PBS	8	0.25 ml/ dose/mouse on day 1	0.25 ml/dose/ mouse starting on day 2	Once every other day (QOD) × 8 weeks; IP

Monitoring of Islet Graft Function

Islet graft function was monitored by serial blood glucose measurements daily for the first 2 weeks after islet transplantation, followed by every other day thereafter. Reversal of diabetes was defined as blood glucose less than 200 mg/dl on two consecutive measurements. Percentage graft loss was determined when blood glucose exceeded 250 mg/dl on two consecutive measurements. Results are shown in Table 7.

TABLE 7

Effects of TTP-4000 and TTP-3000 on Recurrent Diabetes In Syngeneic Islet Transplants In NOD Mice*			
	TTP-4000 300 ug LD + 100 ug qod ip (Group 1)	TTP-3000 300 ug + 100 ug qod ip (Group 2)	CONTROL
	35	44	23
	38	46	25
	40	42	26
	43	41	22
	36	34	22
	45	32	24
	44	30	21
	38		20
			22
			21
			24
Mean	39.875	38.42857	22.727273
SD	3.758324	6.32079	1.8488326
n	8	7	11

*Values reflect the day of graft loss for each animal as defined by recurrence of increased blood glucose levels.

The effects of administering TTP-4000 on rejection of syngeneic transplanted islets in diabetic NOD mice are shown as a Kaplan-Meier Cumulative Survival Plot in FIG. 18. As shown in the data of Table 7, there was an increase in the time before detection of graft failure for animals treated with TTP-4000 (Group 1) and TTP-3000 (Group 2) as opposed to animals that are not treated at all (Control). FIG. 18 shows the increase in time before detection of graft failure for animals treated with TTP-4000 (Group 1) and animals that are not treated at all. Using a variety of statistical analyses (Mantel-Cox Logrank, Breslow-Gehan-Wilcoxon; Tarone-Ware, Peto-Peto-Wilcoxin; Harrington-Fleming) the differences between the Control and TTP 4000 (Group 1) and the Control and TTP-3000 (Group 2) were significant (Table 8).

70

TABLE 8

Statistical Method	Control vs Group 1 (TTP-4000)			Control vs Group 2 (TTP-3000)		
	Chi-Square	DF*	P-value	Chi-Square	DF	P-value
Logrank (Mantel-Cox)	18.410	1	<0.0001	16.480	1	<0.0001
Breslow-Gehan-Wilcoxon	14.690	1	0.0001	12.927	1	0.0001
Tarone-Ware	16.529	1	<0.0001	14.686	1	0.0001
Peto-Peto-Wilcoxon	14.812	1	0.0001	13.027	1	0.0003
Harrington-Fleming (rho = 0.5)	16.529	1	<0.0001	14.686	1	0.0001

*Degrees of Freedom

Example 9

RAGE Fusion Protein Lyophilized Formulation

In the development of a lyophilized formulation using the RAGE fusion protein TTP-4000, lyoprotectants and buffers were initially screened by measuring the stability of the protein after lyophilization and reconstitution. The lyophilized protein in each formulation was also subjected to accelerated stability studies to determine the potential stability of the protein over its shelf-life.

In initial screening studies, experiments were designed to evaluate formulation conditions that could provide appropriate solubility and stability of TTP-4000 formulated as a frozen bulk and provide reconstituted formulations having concentrations of RAGE fusion protein at about 50 mg/mL or higher. Formulations containing sodium acetate, sodium citrate, sodium phosphate, sodium succinate, histidine, and sodium chloride were tested along with sucrose and mannitol. Various pH's between 5.5 and 7.0 were also evaluated.

The biophysical and chemical stability of TTP-4000 was evaluated using size exclusion chromatography (SEC), SDS-Page, Fourier-Transform Infrared Spectroscopy (FTIR), circular dichroism (CD), peptide mapping, and ultra-violet-visible absorbance (UV-Vis).

Based on the solubility of the RAGE fusion protein in formulations containing one or more of sodium citrate, histidine, sucrose, mannitol, and Tween 80 at a pH of 7.0 or less, formulations containing one or more of these buffers, lyoprotectants, or surfactant were selected for further study.

Example 10

RAGE Fusion Protein Lyophilized Formulation

Based on the information collected in Example 9, additional formulations of TTP-4000 were studied. The studies focused on identifying the buffers and/or lyoprotectants useful to maintain a stable product through the freeze-drying process and potentially achieve a high concentration of TTP-4000 upon reconstitution (i.e., at about or above 50 mg/mL). Six formulations were studied during a 2 week accelerated stability study. The formulations are summarized in Table 9. Studies also focused on developing a freeze-drying cycle for larger doses of TTP-4000 (250 mg) as described below.

Following freeze-drying, the formulation development sample vials were crimped and placed into a 40° C. chamber for 2 weeks. The scale-up development samples were stored at 2-8° C. until testing was performed.

Sample Analysis Methods:

The concentration of TTP-4000 in samples was measured by UV-Vis spectrophotometry. An Agilent UV-Vis was used to obtain protein spectra as well as buffer blank spectra. Once acquired, absorbance values were corrected for any light scattering that may occur as a result of any protein aggregation.

Residual moisture was analyzed using a Karl Fisher titration method. Freeze-dried formulation development samples were reconstituted with the appropriate amount of WFI. The time for reconstitution was considered to be the time from when the water was added to the time when there were no visible solids. The pH of each sample was measured after reconstitution using a properly calibrated semi-micro probe.

Size Exclusion Chromatography (SEC) was performed using a TSKgel Super SW2000 column to analyze or monitor the physical stability (degradation and soluble aggregate formation) of TTP-4000 during freeze-drying and storage at accelerating conditions. Samples were injected into an Agilent 1100 series LC fitted with two TSKgel Super SW2000, 4.6×300 mm, 4 μm columns (Tosoh Bioscience, 18674).

To measure the particle count, a 1 mL sample was diluted 20-fold into 20 mL. The sample was degassed by sonication for about 30 seconds, and gently stirred by hand-swirling without introducing bubbles. Three aliquots, each 5 mL in volume, were withdrawn into the light obscuration counter sensor. With the instrument set to cumulative mode, particles were collected at settings of greater or equal to 10 μm and greater or equal to 25 μm.

Results:

Based on the preformulation studies in Example 9, two buffers were initially investigated: sodium citrate and L-histidine. The concentrated formulations 1-6 (Table 9) were prepared using centrifugal concentrators. The final protein concentration in the formulations 1-6 in Table 9 was in a range between about 4-15 mg/mL.

TABLE 9

Formulations of TTP-4000						
	Sodium Citrate (mM)	Histidine (mM)	Sucrose (mM)	Mannitol (mM)	Tween 80 (%)	pH
1	10		60			6.0
2	10		30	50		6.0
3	10		60		0.01	6.0
4		10	60			6.0
5		10	30	50		6.0
6		10	60		0.01	6.0

Using formulations 1-6 in Table 9, vials (2 mL) were used with 0.7 mL fill volumes. The vial headspace was filled with air. Prior to drying, the samples were frozen at a shelf temperature of between -50° C. to -20° C. for approximately 12 hours. The samples were dried at a reduced pressure of 100 mTorr and a shelf temperature of between -20° C. and -10° C. for approximately 36 hours, followed by a shelf temperature of 20° C. for approximately 12 hours. Following freeze drying, the vials were stoppered, and the tops were crimped. Rugged cakes were produced from each formulation 1-6.

The freeze-dried products were subjected to accelerated stability study in order to assess the chemical stability of the formulations. The freeze-dried products were placed in storage at 40° C. and 75% relative humidity for 2 weeks.

The freeze-dried products were reconstituted with 0.206 mL WFI. All freeze-dried products were reconstituted within 20 seconds or less. The pH remained consistent in all reconstituted formulations throughout the 2 week storage period. Residual moisture values determined at times 0 and 2 weeks for the freeze-dried products were between 3.0% and 0.8% and showed that the freeze-drying cycle was able to sufficiently dry the pre-lyophilized formulations. The osmolality of all the reconstituted formulations was within a desirable isotonic range between 250 mOsm/kg and 400 mOsm/kg (See Table 10). The viscosity of each reconstituted formulation was below 3.7 cP (centiPoise).

TABLE 10

Reconstituted formulations of TTP-4000 and osmolality						
	Sodium Citrate (mM)	Histidine (mM)	Sucrose (mM)	Mannitol (mM)	Tween 80 (%)	Osmolality (mOsm/kg)
1	10		60			322
2	10		30	50		385
3	10		60		0.01	324
4		10	60			264
5		10	30	50		336
6		10	60		0.01	262

SEC analysis was performed on samples of formulation 5 in Table 10 taken at various steps of the freeze-drying process and indicated that in these formulations, TTP-4000 was not particularly sensitive to any of the steps of the freeze-drying process based on the consistent low levels of aggregate and breakdown species.

SEC analysis was also performed on the pre-lyophilized formulations prior to freeze-drying as well as the reconstituted formulations at time zero and after a 2 week accelerated stability study. The amount of impurities (aggregate or breakdown product) were consistently low (i.e., below 4%) (Table 11).

TABLE 11

Reconstituted formulations of TTP-4000 and % intact protein							
	Sodium Citrate (mM)	Histidine (mM)	Sucrose (mM)	Mannitol (mM)	Tween 80 (%)	% intact protein (T = 0)	% intact protein (T = 2 wks.)
1	10		60			96.2	97.3
2	10		30	50		96.8	97.3
3	10		60		0.01	96.3	97.2
4		10	60			97.4	97.0
5		10	30	50		97.9	96.9
6		10	60		0.01	97.7	96.9

Peptide mapping revealed no oxidation or deamidation of TTP-4000 due to freeze-drying and storage under accelerated conditions.

SDS-PAGE was run on formulations 1, 3, 4, and 6 and showed that TTP-4000 maintained physical stability throughout the freeze-drying and storage process in these formulations.

To scale-up the freeze-drying process for use with 50 mL lyo vials and a dosage of 250 mg of TTP-4000, samples were prepared by concentrating the solution to 15 mg/mL of TTP-4000 using ultrafiltration and then diafiltering the solution against 10 mM histidine and 65 mM sucrose at pH 6.0. Tween 80 was added to a final amount of 0.01% (vol/vol).

The pre-lyophilized formulation (16.67 mL) was added to each 50 mL vial. The samples were exposed to a freeze-drying cycle where the samples cooled at a shelf temperature of between 5° C. and -5° C. for 30 min, followed by cooling at a shelf temperature of -50° C. for approximately 3 hours. The samples were dried at a reduced pressure of 100 mTorr and a shelf temperature of between -20° C. and -10° C. for approximately 34 hours, followed by a shelf temperature of between 5° C. and 20° C. for approximately 11 hours. Following freeze drying, the vials were stoppered, and the tops were crimped. A pharmaceutically elegant, white cake was produced. The cake appeared rugged and did not lose its structure during handling and storage.

The concentration of the reconstituted sample as determined by absorbance at 280 nm was 40.5 mg/mL of TTP-4000. In additional studies under similar conditions, the concentration of TTP-4000 in the reconstituted sample was consistently about 50 mg/mL.

The reconstituted sample was measured for particulate content at 0, 2, and 6 hours of storage at room temperature. The results in Table 12 show that the reconstituted sample had a low amount of particulate content.

TABLE 12

Number of particles detected in reconstituted sample during storage				
	Particle size	Time = 0	Time = 2 hrs.	Time = 6 hrs.
Particles	>10 µm	562	368	948
per mL	>25 µm	8	16	20
Particles per container	>10 µm	2753	1803	4645
	>25 µm	39	78	98

In summary, TTP-4000 was formulated in citrate and histidine buffers containing one or more of sucrose, mannitol, and Tween 80. Testing showed that formulations containing sodium citrate or histidine had similar performance characteristics and that TTP-4000 may be more soluble in formulations containing histidine.

Formulations containing histidine were focused upon for further scale-up studies.

Following a freeze-drying cycle, the chemical and physical stability of TTP-4000 was evaluated, and no significant differences between the histidine formulations were detected. Also, mannitol was eliminated from the formulation. The final formulation chosen from the formulation development study contained 10 mM histidine, 60 mM sucrose, and 0.01% Tween 80 at about pH 6.0. This formulation demonstrated a superior ability to keep TTP-4000 stable during freeze-drying and storage, and also provided the highest concentration of TTP-4000 during the study. In the scale up study, the sucrose level was raised to 65 mM to adjust the osmolality of the formulation closer to isotonicity. During the scale up study, it was also found that maintaining the pH of the TTP-4000 pre-lyophilized formulation and reconstituted formulation at or near pH 6.0 and less than 6.7 was useful pH to reduce protein precipitation or aggregation.

The foregoing is considered as illustrative only of the principal of the invention. Since numerous modifications and changes will readily occur to those skilled in the art, it is not intended to limit the invention to the exact embodiments shown and described, and all suitable modifications and equivalents falling within the scope of the appended claims are deemed within the present inventive concept.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 57

<210> SEQ ID NO 1

<211> LENGTH: 404

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

Met Ala Ala Gly Thr Ala Val Gly Ala Trp Val Leu Val Leu Ser Leu
1 5 10 15

Trp Gly Ala Val Val Gly Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu
20 25 30

Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg
35 40 45

Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
50 55 60

Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
65 70 75 80

Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu Gly Ile
85 90 95

-continued

Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn
 100 105 110
 Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp
 115 120 125
 Ser Ala Ser Glu Leu Thr Ala Gly Val Pro Asn Lys Val Gly Thr Cys
 130 135 140
 Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu Ser Trp His Leu Asp
 145 150 155 160
 Gly Lys Pro Leu Val Pro Asn Glu Lys Gly Val Ser Val Lys Glu Gln
 165 170 175
 Thr Arg Arg His Pro Glu Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu
 180 185 190
 Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys
 195 200 205
 Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala Leu Arg Thr Ala Pro
 210 215 220
 Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val Gln Leu
 225 230 235 240
 Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro Gly Gly Thr Val Thr
 245 250 255
 Leu Thr Cys Glu Val Pro Ala Gln Pro Ser Pro Gln Ile His Trp Met
 260 265 270
 Lys Asp Gly Val Pro Leu Pro Leu Pro Pro Ser Pro Val Leu Ile Leu
 275 280 285
 Pro Glu Ile Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr
 290 295 300
 His Ser Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser Ile Ser Ile
 305 310 315 320
 Ile Glu Pro Gly Glu Glu Gly Pro Thr Ala Gly Ser Val Gly Gly Ser
 325 330 335
 Gly Leu Gly Thr Leu Ala Leu Ala Leu Gly Ile Leu Gly Gly Leu Gly
 340 345 350
 Thr Ala Ala Leu Leu Ile Gly Val Ile Leu Trp Gln Arg Arg Gln Arg
 355 360 365
 Arg Gly Glu Glu Arg Lys Ala Pro Glu Asn Gln Glu Glu Glu Glu
 370 375 380
 Arg Ala Glu Leu Asn Gln Ser Glu Glu Pro Glu Ala Gly Glu Ser Ser
 385 390 395 400
 Thr Gly Gly Pro

<210> SEQ ID NO 2

<211> LENGTH: 382

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15
 Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30
 Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
 35 40 45
 Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
 50 55 60
 Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met

-continued

65	70	75	80
Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr	85	90	95
Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr	100	105	110
Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr	115	120	125
Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro	130	135	140
Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu	145	150	155
Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg	165	170	175
Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu	180	185	190
Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp	195	200	205
Glu Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly	210	215	220
Gly Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr Cys Glu Val Pro	225	230	235
Ala Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu	245	250	255
Pro Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu Ile Gly Pro Gln	260	265	270
Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro	275	280	285
Gln Glu Ser Arg Ala Val Ser Ile Ser Ile Ile Glu Pro Gly Glu Glu	290	295	300
Gly Pro Thr Ala Gly Ser Val Gly Gly Ser Gly Leu Gly Thr Leu Ala	305	310	315
Leu Ala Leu Gly Ile Leu Gly Gly Leu Gly Thr Ala Ala Leu Leu Ile	325	330	335
Gly Val Ile Leu Trp Gln Arg Arg Gln Arg Arg Gly Glu Glu Arg Lys	340	345	350
Ala Pro Glu Asn Gln Glu Glu Glu Glu Glu Arg Ala Glu Leu Asn Gln	355	360	365
Ser Glu Glu Pro Glu Ala Gly Glu Ser Ser Thr Gly Gly Pro	370	375	380

<210> SEQ ID NO 3

<211> LENGTH: 381

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys	1	5	10	15
Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr	20	25	30	
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro	35	40	45	
Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro	50	55	60	
Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn				

-continued

65	70				75				80						
Arg	Asn	Gly	Lys	Glu	Thr	Lys	Ser	Asn	Tyr	Arg	Val	Arg	Val	Tyr	Gln
				85					90					95	
Ile	Pro	Gly	Lys	Pro	Glu	Ile	Val	Asp	Ser	Ala	Ser	Glu	Leu	Thr	Ala
			100					105					110		
Gly	Val	Pro	Asn	Lys	Val	Gly	Thr	Cys	Val	Ser	Glu	Gly	Ser	Tyr	Pro
		115					120					125			
Ala	Gly	Thr	Leu	Ser	Trp	His	Leu	Asp	Gly	Lys	Pro	Leu	Val	Pro	Asn
	130					135					140				
Glu	Lys	Gly	Val	Ser	Val	Lys	Glu	Gln	Thr	Arg	Arg	His	Pro	Glu	Thr
145					150					155					160
Gly	Leu	Phe	Thr	Leu	Gln	Ser	Glu	Leu	Met	Val	Thr	Pro	Ala	Arg	Gly
				165					170					175	
Gly	Asp	Pro	Arg	Pro	Thr	Phe	Ser	Cys	Ser	Phe	Ser	Pro	Gly	Leu	Pro
			180					185					190		
Arg	His	Arg	Ala	Leu	Arg	Thr	Ala	Pro	Ile	Gln	Pro	Arg	Val	Trp	Glu
		195					200					205			
Pro	Val	Pro	Leu	Glu	Glu	Val	Gln	Leu	Val	Val	Glu	Pro	Glu	Gly	Gly
	210					215					220				
Ala	Val	Ala	Pro	Gly	Gly	Thr	Val	Thr	Leu	Thr	Cys	Glu	Val	Pro	Ala
225						230					235				240
Gln	Pro	Ser	Pro	Gln	Ile	His	Trp	Met	Lys	Asp	Gly	Val	Pro	Leu	Pro
				245					250					255	
Leu	Pro	Pro	Ser	Pro	Val	Leu	Ile	Leu	Pro	Glu	Ile	Gly	Pro	Gln	Asp
			260					265					270		
Gln	Gly	Thr	Tyr	Ser	Cys	Val	Ala	Thr	His	Ser	Ser	His	Gly	Pro	Gln
		275					280					285			
Glu	Ser	Arg	Ala	Val	Ser	Ile	Ser	Ile	Ile	Glu	Pro	Gly	Glu	Glu	Gly
	290					295					300				
Pro	Thr	Ala	Gly	Ser	Val	Gly	Gly	Ser	Gly	Leu	Gly	Thr	Leu	Ala	Leu
305					310					315					320
Ala	Leu	Gly	Ile	Leu	Gly	Gly	Leu	Gly	Thr	Ala	Ala	Leu	Leu	Ile	Gly
				325					330					335	
Val	Ile	Leu	Trp	Gln	Arg	Arg	Gln	Arg	Arg	Gly	Glu	Glu	Arg	Lys	Ala
			340					345					350		
Pro	Glu	Asn	Gln	Glu	Glu	Glu	Glu	Glu	Arg	Ala	Glu	Leu	Asn	Gln	Ser
		355					360					365			
Glu	Glu	Pro	Glu	Ala	Gly	Glu	Ser	Ser	Thr	Gly	Gly	Pro			
	370					375					380				

<210> SEQ ID NO 4

<211> LENGTH: 339

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met	Ala	Ala	Gly	Thr	Ala	Val	Gly	Ala	Trp	Val	Leu	Val	Leu	Ser	Leu
1				5					10					15	
Trp	Gly	Ala	Val	Val	Gly	Ala	Gln	Asn	Ile	Thr	Ala	Arg	Ile	Gly	Glu
			20					25					30		
Pro	Leu	Val	Leu	Lys	Cys	Lys	Gly	Ala	Pro	Lys	Lys	Pro	Pro	Gln	Arg
		35					40					45			
Leu	Glu	Trp	Lys	Leu	Asn	Thr	Gly	Arg	Thr	Glu	Ala	Trp	Lys	Val	Leu
	50					55					60				
Ser	Pro	Gln	Gly	Gly	Gly	Pro	Trp	Asp	Ser	Val	Ala	Arg	Val	Leu	Pro

-continued

Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr
 115 120 125

Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro
 130 135 140

Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu
 145 150 155 160

Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg
 165 170 175

Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu
 180 185 190

Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp
 195 200 205

Glu Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly
 210 215 220

Gly Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr Cys Glu Val Pro
 225 230 235 240

Ala Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu
 245 250 255

Pro Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu Ile Gly Pro Gln
 260 265 270

Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro
 275 280 285

Gln Glu Ser Arg Ala Val Ser Ile Ser Ile Ile Glu Pro Gly Glu Glu
 290 295 300

Gly Pro Thr Ala Gly Ser Val Gly Gly Ser Gly Leu Gly
 305 310 315

<210> SEQ ID NO 6
 <211> LENGTH: 316
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110

Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
 115 120 125

Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
 130 135 140

Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
 145 150 155 160

Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
 165 170 175

-continued

Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
 180 185 190

Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu
 195 200 205

Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly
 210 215 220

Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr Cys Glu Val Pro Ala
 225 230 235 240

Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu Pro
 245 250 255

Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu Ile Gly Pro Gln Asp
 260 265 270

Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro Gln
 275 280 285

Glu Ser Arg Ala Val Ser Ile Ser Ile Ile Glu Pro Gly Glu Glu Gly
 290 295 300

Pro Thr Ala Gly Ser Val Gly Gly Ser Gly Leu Gly
 305 310 315

<210> SEQ ID NO 7
 <211> LENGTH: 94
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
 35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
 50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
 65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg
 85 90

<210> SEQ ID NO 8
 <211> LENGTH: 93
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg
 85 90

-continued

<210> SEQ ID NO 9
 <211> LENGTH: 30
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15
 Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys
 20 25 30

<210> SEQ ID NO 10
 <211> LENGTH: 29
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15
 Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys
 20 25

<210> SEQ ID NO 11
 <211> LENGTH: 98
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala Gly Val Pro Asn
 1 5 10 15
 Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu
 20 25 30
 Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn Glu Lys Gly Val
 35 40 45
 Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr Gly Leu Phe Thr
 50 55 60
 Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg
 65 70 75 80
 Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala
 85 90 95

Leu Arg

<210> SEQ ID NO 12
 <211> LENGTH: 91
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val Gln Leu Val Val
 1 5 10 15
 Glu Pro Glu Gly Gly Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr
 20 25 30
 Cys Glu Val Pro Ala Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp
 35 40 45
 Gly Val Pro Leu Pro Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu
 50 55 60
 Ile Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser
 65 70 75 80
 Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser

-continued

85

90

<210> SEQ ID NO 13
 <211> LENGTH: 101
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15
 Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30
 Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
 35 40 45
 Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
 50 55 60
 Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
 65 70 75 80
 Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
 85 90 95
 Gln Ile Pro Gly Lys
 100

<210> SEQ ID NO 14
 <211> LENGTH: 100
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15
 Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30
 Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45
 Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60
 Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80
 Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95
 Ile Pro Gly Lys
 100

<210> SEQ ID NO 15
 <211> LENGTH: 114
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15
 Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30
 Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
 35 40 45
 Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
 50 55 60

-continued

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
85 90 95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
100 105 110

Ala Gly

<210> SEQ ID NO 16
 <211> LENGTH: 113
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
100 105 110

Gly

<210> SEQ ID NO 17
 <211> LENGTH: 204
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
85 90 95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
100 105 110

Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr
115 120 125

Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro
130 135 140

Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu
145 150 155 160

-continued

Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg
 165 170 175

Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu
 180 185 190

Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln
 195 200

<210> SEQ ID NO 18
 <211> LENGTH: 203
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110

Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
 115 120 125

Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
 130 135 140

Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
 145 150 155 160

Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
 165 170 175

Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
 180 185 190

Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln
 195 200

<210> SEQ ID NO 19
 <211> LENGTH: 229
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
 35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
 50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
 65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr

-continued

85				90				95							
Gln	Ile	Pro	Gly	Lys	Pro	Glu	Ile	Val	Asp	Ser	Ala	Ser	Glu	Leu	Thr
			100						105				110		
Ala	Gly	Val	Pro	Asn	Lys	Val	Gly	Thr	Cys	Val	Ser	Glu	Gly	Ser	Tyr
			115				120						125		
Pro	Ala	Gly	Thr	Leu	Ser	Trp	His	Leu	Asp	Gly	Lys	Pro	Leu	Val	Pro
			130				135				140				
Asn	Glu	Lys	Gly	Val	Ser	Val	Lys	Glu	Gln	Thr	Arg	Arg	His	Pro	Glu
			145			150				155					160
Thr	Gly	Leu	Phe	Thr	Leu	Gln	Ser	Glu	Leu	Met	Val	Thr	Pro	Ala	Arg
			165						170					175	
Gly	Gly	Asp	Pro	Arg	Pro	Thr	Phe	Ser	Cys	Ser	Phe	Ser	Pro	Gly	Leu
			180						185				190		
Pro	Arg	His	Arg	Ala	Leu	Arg	Thr	Ala	Pro	Ile	Gln	Pro	Arg	Val	Trp
		195					200						205		
Glu	Pro	Val	Pro	Leu	Glu	Glu	Val	Gln	Leu	Val	Val	Glu	Pro	Glu	Gly
		210				215					220				
Gly	Ala	Val	Ala	Pro											

<210> SEQ ID NO 20

<211> LENGTH: 228

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Gln	Asn	Ile	Thr	Ala	Arg	Ile	Gly	Glu	Pro	Leu	Val	Leu	Lys	Cys	Lys
1				5					10					15	
Gly	Ala	Pro	Lys	Lys	Pro	Pro	Gln	Arg	Leu	Glu	Trp	Lys	Leu	Asn	Thr
			20				25						30		
Gly	Arg	Thr	Glu	Ala	Trp	Lys	Val	Leu	Ser	Pro	Gln	Gly	Gly	Gly	Pro
		35					40						45		
Trp	Asp	Ser	Val	Ala	Arg	Val	Leu	Pro	Asn	Gly	Ser	Leu	Phe	Leu	Pro
		50				55					60				
Ala	Val	Gly	Ile	Gln	Asp	Glu	Gly	Ile	Phe	Arg	Cys	Gln	Ala	Met	Asn
					70					75					80
Arg	Asn	Gly	Lys	Glu	Thr	Lys	Ser	Asn	Tyr	Arg	Val	Arg	Val	Tyr	Gln
					85				90					95	
Ile	Pro	Gly	Lys	Pro	Glu	Ile	Val	Asp	Ser	Ala	Ser	Glu	Leu	Thr	Ala
			100						105				110		
Gly	Val	Pro	Asn	Lys	Val	Gly	Thr	Cys	Val	Ser	Glu	Gly	Ser	Tyr	Pro
			115				120						125		
Ala	Gly	Thr	Leu	Ser	Trp	His	Leu	Asp	Gly	Lys	Pro	Leu	Val	Pro	Asn
			130				135				140				
Glu	Lys	Gly	Val	Ser	Val	Lys	Glu	Gln	Thr	Arg	Arg	His	Pro	Glu	Thr
			145			150				155					160
Gly	Leu	Phe	Thr	Leu	Gln	Ser	Glu	Leu	Met	Val	Thr	Pro	Ala	Arg	Gly
			165						170					175	
Gly	Asp	Pro	Arg	Pro	Thr	Phe	Ser	Cys	Ser	Phe	Ser	Pro	Gly	Leu	Pro
			180						185				190		
Arg	His	Arg	Ala	Leu	Arg	Thr	Ala	Pro	Ile	Gln	Pro	Arg	Val	Trp	Glu
		195					200						205		
Pro	Val	Pro	Leu	Glu	Glu	Val	Gln	Leu	Val	Val	Glu	Pro	Glu	Gly	Gly
			210			215					220				
Ala	Val	Ala	Pro												

-continued

225

<210> SEQ ID NO 21
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Val Tyr Gln Ile Pro Gly Lys
 1 5

<210> SEQ ID NO 22
 <211> LENGTH: 30
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Thr Ala Pro Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu
 1 5 10 15

Val Gln Leu Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro
 20 25 30

<210> SEQ ID NO 23
 <211> LENGTH: 20
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu
 1 5 10 15

Leu Thr Ala Gly
 20

<210> SEQ ID NO 24
 <211> LENGTH: 5
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Thr Ala Pro Ile Gln
 1 5

<210> SEQ ID NO 25
 <211> LENGTH: 354
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60
 gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg 120
 gcccccaaga aaccaccca gcggtgga tggaaactga acacaggccg gacagaagct 180
 tgaaggtcc tgtctccca gggaggaggc cctgggaca gtgtggctcg tgccttccc 240
 aacggctccc tcttcttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300
 gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctac 354

<210> SEQ ID NO 26
 <211> LENGTH: 369
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

-continued

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta    60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg    120
gcccccaaga aaccaccca gcggtggaa tggaaactga acacaggccg gacagaagct    180
tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc    240
aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag    300
gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt    360
cctggaag                                         369

```

```

<210> SEQ ID NO 27
<211> LENGTH: 408
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 27

```

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta    60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg    120
gcccccaaga aaccaccca gcggtggaa tggaaactga acacaggccg gacagaagct    180
tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc    240
aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag    300
gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt    360
cctggaagc cagaaattgt agattctgcc tctgaactca cggctggt                                         408

```

```

<210> SEQ ID NO 28
<211> LENGTH: 690
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 28

```

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta    60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg    120
gcccccaaga aaccaccca gcggtggaa tggaaactga acacaggccg gacagaagct    180
tggaaggtcc tgtctcccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc    240
aacggctccc tcttccttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag    300
gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt    360
cctggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tccaataag    420
gtggggacat gtgtgtcaga ggggagctac cctgcaggga ctcttagctg gcaacttgat    480
gggaagcccc tgggtgcctaa tgagaagga gtatctgtga aggaacagac caggagacac    540
cctgagacag ggctcttcac actgcagtcg gagctaatgg tgacccagc ccggggagga    600
gatccccgtc ccaccttctc ctgtagcttc agcccaggcc tccccgaca ccgggccttg    660
cgcacagccc ccatccagcc ccgtgtctgg                                         690

```

```

<210> SEQ ID NO 29
<211> LENGTH: 753
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

```

```

<400> SEQUENCE: 29

```

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta    60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg    120

```


-continued

```

gcccccaaga aaccaccca gcggtggaa tggaaactga acacaggccg gacagaagct 180
tggaaagtcc tgttccccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240
aacggctccc tcttcttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300
gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360
cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tcccaataag 420
gtggggacat gtgtgtcaga ggggagctac cctgcaggga ctcttagctg gcaactggat 480
gggaagcccc tgggtgcctaa tgagaaggga gtatctgtga aggaacagac caggagacac 540
cctgagacag ggctcttcac actgcagtcg gagctaatgg tgaccccagc ccggggagga 600
gatccccgtc ccaccttctc ctgtagcttc agcccaggcc tccccgaca ccgggccttg 660
cgcacagccc ccatccagcc ccgtgtctgg gagcctgtgc ctctggagga ggtccaattg 720
gtggtggagc cagaaggtgg agcagtagct cct 753

```

<210> SEQ ID NO 30

<211> LENGTH: 1386

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 30

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg 120
gcccccaaga aaccaccca gcggtggaa tggaaactga acacaggccg gacagaagct 180
tggaaagtcc tgttccccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc 240
aacggctccc tcttcttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300
gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360
cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tcccaataag 420
gtggggacat gtgtgtcaga ggggagctac cctgcaggga ctcttagctg gcaactggat 480
gggaagcccc tgggtgcctaa tgagaaggga gtatctgtga aggaacagac caggagacac 540
cctgagacag ggctcttcac actgcagtcg gagctaatgg tgaccccagc ccggggagga 600
gatccccgtc ccaccttctc ctgtagcttc agcccaggcc tccccgaca ccgggccttg 660
cgcacagccc ccatccagcc ccgtgtctgg gagcctgtgc ctctggagga ggtccaattg 720
gtggtggagc cagaaggtgg agcagtagct cctccgtcag tcttctctt cccccaaaa 780
cccaaggaca ccctcatgat ctcccggacc cctgaggtea catgcgtggt ggtggacgtg 840
agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat 900
gccaagacaa agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtcctc 960
accgtcctgc accaggactg gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa 1020
gccctcccag cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca 1080
caggtgtaca cctgcccccc atcccgggat gagctgacca agaaccaggt cagcctgacc 1140
tgctggtca aaggcttcta tcccagcgac atcgccgtgg agtgggagag caatgggcag 1200
ccggagaaca actacaagac cacgcctccc gtgctggact ccgacggctc cttcttctc 1260
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc 1320
gtgatgcatg aggtcttgea caaccactac acgcagaaga gcctctcct gtctccgggt 1380
aatga 1386

```

-continued

<210> SEQ ID NO 31
 <211> LENGTH: 1041
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 31

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta    60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tggtgctgaa gtgtaagggg    120
gcccccaaga aaccaccca gcggtggaa tggaaactga acacaggccg gacagaagct    180
tggaaaggtcc tgtctccca gggaggaggc ccctgggaca gtgtggctcg tgtccttccc    240
aacggctccc tcttcttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag    300
gcaatgaaca ggaatggaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt    360
cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtcc gtcagtcttc    420
ctcttcccc caaaaccca ggacaccctc atgatctccc ggaccctga ggtcacatgc    480
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggtc cgtggacggc    540
gtggaggtgc ataatgccaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt    600
gtggtcagcg tcctcacct cctgcaccag gactggctga atggcaagga gtacaagtgc    660
aaggtctcca acaaagcct cccagcccc atcgagaaaa ccatctcca agccaaaggg    720
cagccccgag aaccacaggt gtacaccctg cccccatccc gggatgagct gaccaagaac    780
caggtcagcc tgacctgct ggtaaaaggc ttctatccca gcgacatcgc cgtggagtgg    840
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac    900
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac    960
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc   1020
tcctgtctc cgggtaaatg a                                     1041
  
```

<210> SEQ ID NO 32
 <211> LENGTH: 461
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 32

```

Met Ala Ala Gly Thr Ala Val Gly Ala Trp Val Leu Val Leu Ser Leu
1           5           10           15

Trp Gly Ala Val Val Gly Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu
          20           25           30

Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg
          35           40           45

Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
          50           55           60

Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
          65           70           75           80

Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu Gly Ile
          85           90           95

Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn
          100          105          110

Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp
          115          120          125
  
```

-continued

Ser Ala Ser Glu Leu Thr Ala Gly Val Pro Asn Lys Val Gly Thr Cys
 130 135 140
 Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu Ser Trp His Leu Asp
 145 150 155 160
 Gly Lys Pro Leu Val Pro Asn Glu Lys Gly Val Ser Val Lys Glu Gln
 165 170 175
 Thr Arg Arg His Pro Glu Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu
 180 185 190
 Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys
 195 200 205
 Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala Leu Arg Thr Ala Pro
 210 215 220
 Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val Gln Leu
 225 230 235 240
 Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro Pro Ser Val Phe Leu
 245 250 255
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 260 265 270
 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 275 280 285
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 290 295 300
 Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 305 310 315 320
 Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 325 330 335
 Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 340 345 350
 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 355 360 365
 Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 370 375 380
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 385 390 395 400
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 405 410 415
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 420 425 430
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 435 440 445
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 450 455 460

<210> SEQ ID NO 33

<211> LENGTH: 439

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 33

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15
 Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30
 Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly

-continued

35					40					45					
Pro	Trp	Asp	Ser	Val	Ala	Arg	Val	Leu	Pro	Asn	Gly	Ser	Leu	Phe	Leu
50					55					60					
Pro	Ala	Val	Gly	Ile	Gln	Asp	Glu	Gly	Ile	Phe	Arg	Cys	Gln	Ala	Met
65					70					75					80
Asn	Arg	Asn	Gly	Lys	Glu	Thr	Lys	Ser	Asn	Tyr	Arg	Val	Arg	Val	Tyr
				85					90					95	
Gln	Ile	Pro	Gly	Lys	Pro	Glu	Ile	Val	Asp	Ser	Ala	Ser	Glu	Leu	Thr
			100					105					110		
Ala	Gly	Val	Pro	Asn	Lys	Val	Gly	Thr	Cys	Val	Ser	Glu	Gly	Ser	Tyr
			115				120					125			
Pro	Ala	Gly	Thr	Leu	Ser	Trp	His	Leu	Asp	Gly	Lys	Pro	Leu	Val	Pro
						135					140				
Asn	Glu	Lys	Gly	Val	Ser	Val	Lys	Glu	Gln	Thr	Arg	Arg	His	Pro	Glu
145					150					155					160
Thr	Gly	Leu	Phe	Thr	Leu	Gln	Ser	Glu	Leu	Met	Val	Thr	Pro	Ala	Arg
				165					170					175	
Gly	Gly	Asp	Pro	Arg	Pro	Thr	Phe	Ser	Cys	Ser	Phe	Ser	Pro	Gly	Leu
			180					185					190		
Pro	Arg	His	Arg	Ala	Leu	Arg	Thr	Ala	Pro	Ile	Gln	Pro	Arg	Val	Trp
		195					200					205			
Glu	Pro	Val	Pro	Leu	Glu	Glu	Val	Gln	Leu	Val	Val	Glu	Pro	Glu	Gly
						215					220				
Gly	Ala	Val	Ala	Pro	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys
225					230					235					240
Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val
				245					250					255	
Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp
			260					265					270		
Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr
		275					280					285			
Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp
		290					295					300			
Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu
305					310					315					320
Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg
				325					330					335	
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys
			340					345					350		
Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp
		355					360					365			
Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys
						375					380				
Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser
385					390					395					400
Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser
				405					410					415	
Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser
			420					425					430		
Leu	Ser	Leu	Ser	Pro	Gly	Lys									
				435											

<210> SEQ ID NO 34

<211> LENGTH: 438

-continued

<212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificial = synthetic construct

 <400> SEQUENCE: 34

 Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15
 Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30
 Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45
 Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60
 Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80
 Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95
 Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110
 Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
 115 120 125
 Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
 130 135 140
 Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
 145 150 155 160
 Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
 165 170 175
 Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
 180 185 190
 Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu
 195 200 205
 Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly
 210 215 220
 Ala Val Ala Pro Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 225 230 235 240
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 245 250 255
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 260 265 270
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
 275 280 285
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 290 295 300
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 305 310 315 320
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
 325 330 335
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
 340 345 350
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 355 360 365
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 370 375 380
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys

-continued

385	390	395	400
Leu Thr Val Asp	Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys		
	405	410	415
Ser Val Met His	Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu		
	420	425	430
Ser Leu Ser Pro Gly Lys			
	435		
<210> SEQ ID NO 35 <211> LENGTH: 346 <212> TYPE: PRT <213> ORGANISM: Artificial <220> FEATURE: <223> OTHER INFORMATION: Artificial = synthetic construct <400> SEQUENCE: 35			
Met Ala Ala Gly Thr Ala Val Gly Ala Trp Val Leu Val Leu Ser Leu			
1	5	10	15
Trp Gly Ala Val Val Gly Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu			
	20	25	30
Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg			
	35	40	45
Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu			
50	55	60	
Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro			
65	70	75	80
Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu Gly Ile			
	85	90	95
Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn			
	100	105	110
Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp			
	115	120	125
Ser Ala Ser Glu Leu Thr Ala Gly Pro Ser Val Phe Leu Phe Pro Pro			
	130	135	140
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys			
145	150	155	160
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp			
	165	170	175
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu			
	180	185	190
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu			
	195	200	205
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn			
	210	215	220
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly			
225	230	235	240
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu			
	245	250	255
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr			
	260	265	270
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn			
	275	280	285
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe			
	290	295	300
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn			
305	310	315	320

-continued

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 325 330 335

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 340 345

<210> SEQ ID NO 36

<211> LENGTH: 324

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 36

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
 1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
 20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
 35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
 50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
 65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
 85 90 95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
 100 105 110

Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 115 120 125

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 130 135 140

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 145 150 155 160

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 165 170 175

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 180 185 190

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 195 200 205

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 210 215 220

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
 225 230 235 240

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 245 250 255

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 260 265 270

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 275 280 285

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 290 295 300

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 305 310 315 320

Ser Pro Gly Lys

-continued

```

<210> SEQ ID NO 37
<211> LENGTH: 323
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 37

Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1          5          10          15
Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20          25          30
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35          40          45
Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50          55          60
Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65          70          75          80
Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
85          90          95
Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
100         105         110
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
115         120         125
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
130         135         140
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
145         150         155         160
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
165         170         175
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
180         185         190
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
195         200         205
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
210         215         220
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
225         230         235         240
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
245         250         255
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
260         265         270
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
275         280         285
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
290         295         300
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
305         310         315         320

Pro Gly Lys

```

```

<210> SEQ ID NO 38
<211> LENGTH: 210
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

```

```

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile

```


-continued

1	5	10	15
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu	20	25	30
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His	35	40	45
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg	50	55	60
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys	65	70	75
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu	85	90	95
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr	100	105	110
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu	115	120	125
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp	130	135	140
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val	145	150	155
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp	165	170	175
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His	180	185	190
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro	195	200	205
Gly Lys	210		

<210> SEQ ID NO 39
 <211> LENGTH: 633
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 39

```

ccgtcagtct tctcttccc cccaaaaccc aaggacaccc tcatgatctc cggaccct 60
gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 120
tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 180
agcacgtacc gtgtggtcag cgctctcacc gtcctgcacc aggactggct gaatggcaag 240
gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 300
aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag 360
ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 420
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 480
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 540
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccaactacag 600
cagaagagcc tctcctgtc tccgggtaaa tga 633
    
```

<210> SEQ ID NO 40
 <211> LENGTH: 220
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe

-continued

1	5	10	15
Pro	Pro Lys	Pro Lys Asp Thr Leu Met	Ile Ser Arg Thr Pro Glu Val
	20	25	30
Thr	Cys Val Val Val Asp Val Ser	His Glu Asp Pro Glu Val Lys Phe	
	35	40	45
Asn	Trp Tyr Val Asp Gly Val Glu Val His Asn	Ala Lys Thr Lys Pro	
	50	55	60
Arg	Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr		
	65	70	75 80
Val	Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val		
	85	90	95
Ser	Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala		
	100	105	110
Lys	Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg		
	115	120	125
Asp	Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly		
	130	135	140
Phe	Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro		
	145	150	155 160
Glu	Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser		
	165	170	175
Phe	Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln		
	180	185	190
Gly	Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His		
	195	200	205
Tyr	Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		
	210	215	220

<210> SEQ ID NO 41
 <211> LENGTH: 663
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

```

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc 60
aaggacaccc tcatgatctc cgggaccct gaggtcacat gcgtggtggt ggacgtgagc 120
cacgaagacc ctgaggtcaa gttcaactgg tacgtggagc gcgtggaggt gcataatgcc 180
aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 240
gtcctgcacc aggactggct gaatggcaag gactacaagt gcaaggtctc caacaaagcc 300
ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 360
gtgtacaccc tgccccatc cgggatgag ctgaccaaga accaggtcag cctgacctgc 420
ctgggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 480
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt ctctctctac 540
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 600
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 660
tga 663
    
```

<210> SEQ ID NO 42
 <211> LENGTH: 113
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

-continued

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 1 5 10 15
 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 20 25 30
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 35 40 45
 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 50 55 60
 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 65 70 75 80
 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 85 90 95
 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 100 105 110

Lys

<210> SEQ ID NO 43
 <211> LENGTH: 107
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 43

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 1 5 10 15
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 20 25 30
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 35 40 45
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 50 55 60
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 65 70 75 80
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 85 90 95
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 100 105

<210> SEQ ID NO 44
 <211> LENGTH: 25
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 44

Ile Ser Ile Ile Glu Pro Gly Glu Glu Gly Pro Thr Ala Gly Ser Val
 1 5 10 15
 Gly Gly Ser Gly Leu Gly Thr Leu Ala
 20 25

<210> SEQ ID NO 45
 <211> LENGTH: 316
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 45

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys

-continued

1	5	10	15
Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr	20	25	30
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro	35	40	45
Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro	50	55	60
Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn	65	70	75
Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln	85	90	95
Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala	100	105	110
Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro	115	120	125
Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn	130	135	140
Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr	145	150	155
Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly	165	170	175
Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro	180	185	190
Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu	195	200	205
Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly	210	215	220
Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr Cys Glu Val Pro Ala	225	230	235
Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu Pro	245	250	255
Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu Ile Gly Pro Gln Asp	260	265	270
Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro Gln	275	280	285
Glu Ser Arg Ala Val Ser Ile Ser Ile Ile Glu Pro Gly Glu Glu Gly	290	295	300
Pro Thr Ala Gly Ser Val Gly Gly Ser Gly Leu Gly	305	310	315

<210> SEQ ID NO 46

<211> LENGTH: 93

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (1)..(1)

<223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 46

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys	1	5	10	15
Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr	20	25	30	
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro	35	40	45	

-continued

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg
 85 90

<210> SEQ ID NO 47
 <211> LENGTH: 29
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 47

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys
 20 25

<210> SEQ ID NO 48
 <211> LENGTH: 100
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 48

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95

Ile Pro Gly Lys
 100

<210> SEQ ID NO 49
 <211> LENGTH: 113
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 49

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45

-continued

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60
 Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80
 Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95
 Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110

Gly

<210> SEQ ID NO 50
 <211> LENGTH: 203
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 50

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15
 Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30
 Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45
 Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60
 Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80
 Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95
 Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110
 Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
 115 120 125
 Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
 130 135 140
 Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
 145 150 155 160
 Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
 165 170 175
 Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
 180 185 190
 Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln
 195 200

<210> SEQ ID NO 51
 <211> LENGTH: 228
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 51

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
 1 5 10 15

-continued

Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30

Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45

Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
 50 55 60

Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
 65 70 75 80

Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95

Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110

Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro
 115 120 125

Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn
 130 135 140

Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr
 145 150 155 160

Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly
 165 170 175

Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro
 180 185 190

Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu
 195 200 205

Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly
 210 215 220

Ala Val Ala Pro
 225

<210> SEQ ID NO 52
 <211> LENGTH: 633
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 52

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccct 60
 gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 120
 tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 180
 agcaegtacc gtgtggtcag cgtcctcacc gtctgcacc aggactggct gaatggcaag 240
 gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 300
 aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag 360
 ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gttctatcc cagcgacatc 420
 gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 480
 ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 540
 cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccaactacag 600
 cagaagagcc tctcctgtc tcccgggaaa tga 633

<210> SEQ ID NO 53
 <211> LENGTH: 663
 <212> TYPE: DNA
 <213> ORGANISM: Artificial

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 53

```

ccgtgcccag cacctgaact cctgggggga cgcgcagtct tcctcttccc cccaaaaccc 60
aaggacaccc tcatgatctc cgggaccctc gaggtcacat gcgtgggtgt ggacgtgagc 120
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 180
aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtgggtcag cgtcctcacc 240
gtcctgcacc aggactggct gaatggcaag gactacaagt gcaaggtctc caacaaagcc 300
ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 360
gtgtacaccc tgccccatc cgggatgag ctgaccaaga accaggtcag cctgacctgc 420
ctgggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 480
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt ctctctctac 540
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 600
atgcatgagg ctctgcacaa ccaactacag cagaagagcc tctccctgtc tcccgggaaa 660
tga 663

```

<210> SEQ ID NO 54

<211> LENGTH: 1386

<212> TYPE: DNA

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Artificial = synthetic construct

<400> SEQUENCE: 54

```

atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg 120
gcccccaaga aaccaccca gcggttgaa tggaaactga acacaggccg gacagaagct 180
tggaaggtcc tgtctcccca gggaggagcc cctgggaca gtgtggctcg tgtccttccc 240
aacggctccc tcttcttccc ggetgtcggg atccaggatg aggggatctt ccggtgccag 300
gcaatgaaca ggaatgaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360
cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtgt tccaataag 420
gtggggacat gtgtgtcaga ggggagctac cctgcagggg ctcttagctg gcaactggat 480
gggaagcccc tgggtgcctaa tgagaagggg gtatctgtga aggaacagac caggagacac 540
cctgagacag ggctcttcac actgcagtcg gagctaattg tgaccccagc ccggggagga 600
gatccccgct ccaccttctc ctgtagcttc agcccaggcc tccccgaca ccgggccttg 660
cgcacagccc ccatccagcc ccgtgtctgg gagcctgtgc ctctggagga ggtccaattg 720
gtgggtggagc cagaaggtgg agcagtagct cctccgtcag tcttctctt cccccaaaa 780
cccaaggaca ccctcatgat ctcccggacc cctgaggtca catgctggtt ggtggacgtg 840
agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat 900
gccaagacaa agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtcctc 960
accgtcctgc accaggactg gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa 1020
gccctcccag cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca 1080
caggtgtaca ccctgcccc atcccgggat gagctgacca agaaccaggt cagcctgacc 1140
tgctgtgca aaggcttcta tcccagcagc atcgcctgg agtgggagag caatgggcag 1200
ccggagaaca actacaagac cagcctccc gtgctggact ccgacggctc cttcttctc 1260

```


-continued

```
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtett ctcatgctcc 1320
gtgatgcatg aggctctgca caaccactac acgcagaaga gcctctccct gtctcccggg 1380
aatga 1386
```

```
<210> SEQ ID NO 55
<211> LENGTH: 1041
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificial = synthetic construct
```

```
<400> SEQUENCE: 55
```

```
atggcagccg gaacagcagt tggagcctgg gtgctggtcc tcagtctgtg gggggcagta 60
gtaggtgctc aaaacatcac agcccggatt ggcgagccac tgggtgctgaa gtgtaagggg 120
gcccccaaga aaccaccca gcggttgaa tggaaactga acacaggccg gacagaagct 180
tggaaggctc tgtctcccca gggaggagcc cctgggaca gtgtggctcg tgtccttccc 240
aacggctccc tcttcttcc ggctgtcggg atccaggatg aggggatttt ccggtgccag 300
gcaatgaaca ggaatgaaa ggagaccaag tccaactacc gagtccgtgt ctaccagatt 360
cctgggaagc cagaaattgt agattctgcc tctgaactca cggctggtcc gtcagtcttc 420
ctcttcccc caaaaccca ggacaccctc atgatctccc ggaccctga ggtcacatgc 480
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 540
gtggaggctc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 600
gtggctcagc tctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 660
aaggtctcca acaaagcct cccagcccc atcgagaaaa ccatctcaa agccaaaggg 720
cagccccgag aaccacaggt gtacaccctg cccccatccc gggatgagct gaccaagaac 780
caggtcagcc tgacctgct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 840
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 900
ggctccttct tctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 960
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1020
tcctgtctc ccgggaaatg a 1041
```

```
<210> SEQ ID NO 56
<211> LENGTH: 438
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificial = synthetic construct
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa=pyroglutamic acid
```

```
<400> SEQUENCE: 56
```

```
Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1           5           10           15
Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
20           25           30
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
35           40           45
Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50           55           60
Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
```

-continued

65	70	75	80
Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln	85	90	95
Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala	100	105	110
Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr Pro	115	120	125
Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro Asn	130	135	140
Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu Thr	145	150	155
Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg Gly	165	170	175
Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu Pro	180	185	190
Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp Glu	195	200	205
Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly Gly	210	215	220
Ala Val Ala Pro Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp	225	230	235
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp	245	250	255
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly	260	265	270
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn	275	280	285
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp	290	295	300
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro	305	310	315
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu	325	330	335
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn	340	345	350
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile	355	360	365
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr	370	375	380
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys	385	390	395
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys	405	410	415
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu	420	425	430
Ser Leu Ser Pro Gly Lys	435		

<210> SEQ ID NO 57

<211> LENGTH: 323

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Artificial = synthetic construct

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

-continued

<222> LOCATION: (1)..(1)

<223> OTHER INFORMATION: Xaa=pyroglutamic acid

<400> SEQUENCE: 57

Xaa Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys Lys
1 5 10 15
Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn Thr
 20 25 30
Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly Pro
 35 40 45
Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu Pro
50 55 60
Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met Asn
65 70 75 80
Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr Gln
 85 90 95
Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr Ala
 100 105 110
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 115 120 125
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
130 135 140
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
145 150 155 160
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 165 170 175
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 180 185 190
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 195 200 205
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
210 215 220
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
225 230 235 240
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 245 250 255
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 260 265 270
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 275 280 285
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
290 295 300
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
305 310 315 320
Pro Gly Lys

What is claimed is:

1. An isolated DNA molecule having the sequence as set forth in SEQ ID NO: 54.

2. An isolated DNA molecule having the sequence as set forth in SEQ ID NO: 55.

3. An expression vector comprising the sequence as set forth in SEQ ID NO: 54.

4. An expression vector comprising the sequence as set forth in SEQ ID NO: 55.

60 5. A cell transfected with an expression vector of claim 3 or claim 4.

* * * * *